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PROCEEDINGS
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*PROPAGATION OF SPHERICAL SHOCK WAVES IN STELLAR INTERIORS**

By Zdeněk Kopal and C. C. Lin

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Communicated by Harlow Shapley, June 28, 1951

1. Introduction.—It has been recognized now for some time[1] that the shock waves in the interiors of white subdwarfs, originating as a result of sudden disturbances of as yet unknown nature, are probably responsible for some of the most spectacular features of observed Nova outbursts. Owing to the mathematical difficulties of the underlying dynamical problem, however, a consistent rational theory of the phenomenon relating the observed boundary phenomena with specific initial conditions is still almost entirely lacking, and the applications of the existing shock wave theory to the Nova outbursts have so far scarcely exceeded the qualitative stage. On the other hand, some recent work on the propagation of spherical shock waves through gravitating gas spheres by one of us[2] concerned the construction of solutions which are possible only in certain definite models whose equilibrium properties are rather far from the expected internal structure of white subdwarfs.[3]

There seems, in brief, indeed no room for doubt that the solutions of the actual astrophysical problem, starting from proper initial conditions, will defy any analytical approach and can be obtained only by a step-by-step process of numerical integration of a certain system of non-linear partial differential equations, with boundary conditions given over two moving surfaces. The aim of the present paper will, therefore, be twofold: to formulate such systems of differential equations which, in our opinion, cannot be simplified further without risking the suppression of some essential features of the phenomenon which they are to describe, and to reduce them to the form in which they can be made amenable to numerical integration over a finite characteristic mesh. It is the difficulty inherent in this latter process, and in the proper application of the boundary conditions, which is probably responsible for the fact that no such solutions have so far ever been attempted by any investigator.

2. *Fundamental Equations of the Problem.*—Let, in what follows, u, p and ρ denote the radial velocity, pressure and density of a compressible fluid at a distance r from the origin, and at a time t. If, moreover, m denotes the mass interior to r, and G, the gravitation constant, the Eulerian hydrodynamical equations governing a spherically symmetrical flow of gas under the influence of its own gravitation reduce to

$$\frac{\partial u}{\partial t} + u\frac{\partial u}{\partial r} + \frac{1}{\rho}\frac{\partial p}{\partial r} + \frac{Gm}{r^2} = 0, \tag{1}$$

where

$$\frac{\partial m}{\partial r} = 4\pi\rho r^2. \tag{2}$$

The equation of continuity can be expressed as

$$\frac{\partial \rho}{\partial t} + u\frac{\partial \rho}{\partial r} + \rho\frac{\partial u}{\partial r} + \frac{2u\rho}{r} = 0. \tag{3}$$

The energy equation appropriate for a gas at high temperatures prevailing in stellar interiors, in which nuclear energy is being liberated at a rate of ϵ ergs per unit mass assumes the form[4]

$$\frac{\partial p}{\partial t} - w_*^2\frac{\partial \rho}{\partial t} + u\left(\frac{\partial p}{\partial r} - w_*^2\frac{\partial \rho}{\partial r}\right) + a\left(\gamma - \frac{4}{3}\right)\rho\left(\frac{\partial}{\partial t} + u\frac{\partial}{\partial r}\right)\frac{T^4}{\rho} = (\gamma - 1)\rho\left(\epsilon - \left(\frac{\partial S}{\partial m}\right)_t\right), \tag{4}$$

where γ denotes the ratio of specific heats of stellar material (which will hereafter be taken as a constant); a, the Stefan constant; $w_*^2 = \gamma p/\rho$ stands for the local velocity of sound; T, for the absolute temperature; and S, for the net flux of radiation through a sphere of radius r, which is defined by

$$S = (4\pi r^2)^2\frac{4acT^3}{3\kappa(\rho, T)}\left(\frac{\partial T}{\partial m}\right)_t, \tag{5}$$

where c is the velocity of light and $\kappa(\rho, T)$, the coefficient of opacity, per unit mass, of stellar matter. In these equations, the total pressure p is a sum of the gas pressure and the radiation pressure, of the form

$$p = \frac{R}{\mu}\rho T + \frac{1}{3}aT^4, \tag{6}$$

where R denotes the gas constant and μ, the molecular weight.

Provided that the functions $\epsilon(\rho, T)$ and $\kappa(\rho, T)$ are known, say, from the

atomic theory, equations (1) to (4) should permit us, in principle, to solve for u, p, ρ and m (or, rather, u, ρ, T and m) in terms of the independent variables r and t. It is, however, possible, and in some respects advantageous, to interchange the roles of m and r by adopting the Lagrangian point of view, which permits us to render r as well as u, p and ρ dependent on t and the initial value of m at the time $t = 0$. Doing so, we find the equivalent system of the Lagrangian equations of motion of our problem to assume the form

$$\frac{\partial u}{\partial t} + 4\pi r^2 \frac{\partial p}{\partial m} + \frac{Gm}{r^2} = 0, \tag{7}$$

where

$$u = \frac{\partial r}{\partial t}; \tag{8}$$

the equation of continuity can now be written as

$$\frac{\partial r}{\partial m} = \frac{1}{4\pi\rho r^2}. \tag{9}$$

The energy equation becomes

$$\frac{\partial p}{\partial t} - {w_*}^2 \frac{\partial \rho}{\partial t} + a\left(\gamma - \frac{4}{3}\right)\rho \frac{\partial}{\partial t}\left(\frac{T^4}{\rho}\right) = \rho(\gamma - 1)\left\{\epsilon - \frac{\partial S}{\partial m}\right\}. \tag{10}$$

The foregoing system of equations (7) to (10) defines the dependent variables r, u, ρ and T as functions of the time t and the mass m contained within a sphere of the radius r_0 describing the initial position of the particle under consideration; the relation between the Lagrangian variable m and the initial position r_0 of any particle being provided by

$$\frac{dm}{dr_0} = 4\pi\rho_0 r_0^2, \tag{11}$$

where $\rho_0(r_0)$ denotes the initial distribution of density in the undisturbed sphere.

Before proceeding to discuss a solution of the fundamental equations (1) to (4) or (7) to (10) in the Eulerian or Lagrangian form, we wish to point out some simplifications which seem legitimate on the basis of the following physical considerations. First, the radiation pressure $^1/_3\, aT^4$ is known to participate appreciably in the total pressure p only in stars of masses considerably greater than that of the sun; for stars of smaller masses the ratio of the radiation to gas pressure becomes negligible. The masses of white subdwarfs as a class are not yet known with any accuracy; but the absolute magnitudes of the post-Novae combined with the empirical mass-luminos-

ity relation would point to masses which are generally inferior to that of the sun. If so, then, the effects of radiation pressure (i.e., all terms multiplied by the Stefan constant) in equation (6), as well as on the left-hand sides of (4) and (10), can be safely ignored (at least for ordinary Novae).

A further simplification can be obtained if we recall that the initial conditions of our problem (i.e., the structure of the undisturbed star) involves usually the assumption that the pre-Nova is through most part of its interior (if not wholly) in radiative equilibrium, which is defined by

$$\frac{\partial S}{\partial m} = \epsilon.$$

Before any explosion takes place, therefore, the right-hand side of equations (4) and (10) is accordingly equal to zero. After the explosion, the radiative equilibrium will theoretically break down both behind, and in front of, the shock wave (since the instantaneous flux of radiation may, in general, overtake the shock). But—and this is significant—the consequences of any breakdown of radiative equilibrium will make themselves felt on a time scale which is utterly long in comparison with that of a Nova outburst,[5] so

TABLE 1

PHYSICAL VARIABLE	UNIT	NORMALIZED VARIABLE
Length, r	R_0	x
Density, ρ	ρ_0	σ
Pressure, p	$2\pi G\rho_0^2 R_0^2$	π
Mass, m	$4\pi\rho_0 R_0^3$	μ
Time, t	$(2\pi G\rho_0)^{-1/2}$	τ
Velocity, u	$(2\pi G\rho_0 R_0^2)^{1/2}$	u

that during the first few hours following the initial explosion the equilibrium of the regions in front of the shock wave will not be appreciably disturbed. Behind the shock wave, the radiative equilibrium breaks down immediately and gives way to a convective one, for which the right-hand sides of equations (4) and (10) become naturally equal to zero. In what follows, therefore, we shall replace the full-dress equations (4) or (10) expressing the conservation of energy by their simpler forms appropriate for polytropic gas in convective equilibrium.

The preceding considerations exhaust the list of permissible simplifications which we feel justified in introducing without suppressing any essential feature of our problem. Before proceeding, however, with the solution of our fundamental equations, we wish to normalize our variables so as to convert our systems to non-dimensional form. Suppose that we decide to accomplish this by the choice of units shown in table 1, where R_0 and ρ_0 denote arbitrary radius and density which may (though not necessarily so) be identified with the radius of the undisturbed configuration, and with its central density.

Introducing the new non-dimensional variables in the fundamental equations of our problem, the Eulerian equation (1) to (4) will asssume the forms

$$\frac{\partial u}{\partial \tau} + u\frac{\partial u}{\partial x} + \frac{1}{\sigma}\frac{\partial \pi}{\partial x} + \frac{2\mu}{x^2} = 0, \tag{12}$$

$$\frac{\partial \sigma}{\partial \tau} + u\frac{\partial \sigma}{\partial x} + \sigma\left(\frac{\partial u}{\partial x} + \frac{2u}{x}\right) = 0, \tag{13}$$

$$\frac{\partial \pi}{\partial \tau} + u\frac{\partial \pi}{\partial x} - w^2\left(\frac{\partial \sigma}{\partial \tau} + u\frac{\partial \sigma}{\partial x}\right) = 0, \tag{14}$$

$$\frac{\partial \mu}{\partial x} - \sigma x^2 = 0, \tag{15}$$

where

$$w^2 = \gamma\frac{\pi}{\sigma}. \tag{16}$$

In the following discussions, it will be found convenient to replace (15) by

$$\frac{\partial \mu}{\partial \tau} + u\frac{\partial \mu}{\partial x} = 0 \tag{17}$$

through the use of (13). The Lagrangian equations likewise become

$$\frac{\partial u}{\partial \tau} + x^2\frac{\partial \pi}{\partial \mu} + \frac{2\mu}{x^2} = 0, \tag{18}$$

$$\frac{\partial \sigma}{\partial \tau} + \sigma^2 x^2\frac{\partial u}{\partial \mu} + \frac{2\sigma u}{x} = 0, \tag{19}$$

$$\frac{\partial \pi}{\partial \tau} - w^2\frac{\partial \sigma}{\partial \tau} = 0, \tag{20}$$

$$\frac{\partial x}{\partial \tau} - u = 0. \tag{21}$$

3. *Characteristic Equations.*—In a problem of wave propagation, such as the one being considered here, one expects, in general, to find a system of totally hyperbolic differential equations, which can be solved by step-by-step integration; but when there are more than two unknown quantities, the process may be rather complicated.[6] It is, however, sometimes possible to arrange the compatibility relations along the characteristics so that only two of the unknowns appear in differential form. The process of integration is then not much more complicated than in the case of two unknowns

This is indeed possible in the present case by a scheme used previously in a similar problem.[7] It will become clear that this possibility is closely related to the fact that pressure and velocity disturbances are propagated with the velocity of sound relative to the moving stream, while entropy changes can be traced by following the motion of individual fluid particles. To accomplish this transformation, one eliminates the derivatives of σ from (13) by the use of (14). The resultant equation

$$\frac{\partial \pi}{\partial \tau} + u\frac{\partial \pi}{\partial x} + \gamma\pi\left(\frac{\partial u}{\partial x} + \frac{2u}{x}\right) = 0 \tag{22}$$

involves only the derivatives of π and u, just as in (12). Thus, one may expect to find, associated with (12) and (22), two characteristic directions, along which differential relations involving only $d\pi$ and du (and $d\tau$) hold. Indeed, by considering the system of equations (12), (22), (14) and (17), one finds that, associated with the first two equations, there are the relations

$$\frac{d\pi}{\gamma\pi} \pm \frac{du}{w} = -\left(\frac{2u}{x} \pm \frac{2\mu}{wx^2}\right)d\tau, \text{ along } dx = (u \pm w)d\tau, \tag{23}$$

associated with (14), there is the relation

$$d\pi - w^2 d\sigma = 0, \qquad \text{along } dx = ud\tau; \tag{24}$$

and associated with (17), we have

$$d\mu = 0, \qquad \text{along } dx = ud\tau. \tag{25}$$

Thus, the path line appears as a double characteristic.

In the Lagrangian system, a similar treatment is possible. One can easily eliminate $\partial\sigma/\partial\tau$ from (19) and obtain

$$\frac{\partial \pi}{\partial \tau} + w^2\sigma^2 x^2\frac{\partial u}{\partial \mu} + \frac{2\sigma u w^2}{x} = 0 \tag{26}$$

so that (18) and (26) involve only derivatives of u and π. The systems (18), (19), (20) and (21) lead to the following system of differential relations for the characteristics:

$$\frac{d\pi}{\gamma\pi} \pm \frac{du}{w} = -\left(\frac{2u}{x} \pm \frac{2\mu}{wx^2}\right)d\tau \qquad \text{along } d\mu = \pm \sigma x^2 w d\tau, \tag{27}$$

$$d\pi - w^2 d\sigma = 0 \qquad \text{along } d\mu = 0, \tag{28}$$

$$dx = ud\tau \qquad \text{along } d\mu = 0. \tag{29}$$

Notice that equations (28) and (29) are exactly the same as (24) and (25) except for a rearrangement of the order of the equations. The first equation in (27) is also identical with that in (23). The second equation in (27) can

be easily derived from (17) by using the definition

$$\mu = \int_0^x \sigma x^2 \, dx$$

and calculating the total derivative $d\mu/d\tau$ along $dx/d\tau = u \pm w$. Thus, the above results can be summarized in the following table in which the difference in the choice of coordinate systems disappears.

In the process of numerical integration, the differentials in the above relations are to be replaced by finite differences. With data known along an initial curve C (fig. 1), the values of the six variables x, τ, π, u, σ, μ are sought in its domain of influence. If we use (x, τ) as the independent variables (Eulerian), their values at P_3 are first calculated from known data at P_1, P_2 by the use of (A); those of π and u by (B). The path line P_0P_3 may then be determined by (D), and the values of σ and μ are determined by using (E) and (F). The relation (C) may be used as a check. If (μ, τ) are taken as independent variables (Lagrangian), their values at P_3 are first determined from the known data at P_1, P_2 by the use of (C); those of π and u by (B). The path line P_0P_3 is given by (F) to be μ = const., and the values of x and σ are then determined by the use of (D) and (E), respectively. The relations (A) may be used for checking. As can be easily seen from table 2, one advantage of the Lagrangian coordinate system is the fact that the path lines are directly integrable.

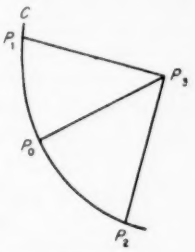

FIGURE 1

4. *Boundary Conditions.*—For sufficiently strong central explosions, the radial flow governed by the equations which were set forth in preceding sections will be separated from the medium in front (which may be at rest, or moving) by a shock wave, at which the radial velocity, pressure and density undergo a discontinuous change. If subscripts 0 and 1 are hereafter used to denote the respective properties in front of, and immediately behind, the advancing shock front, the conservation of mass, energy and momentum across the shock can be shown[8] to require that

$$\frac{u_1 - u_0}{w_0} = \frac{2M}{\gamma + 1}\left(1 - \frac{1}{M^2}\right), \tag{30}$$

$$\frac{\pi_1}{\pi_0} = \frac{2\gamma}{\gamma + 1} M^2 - \frac{\gamma - 1}{\gamma + 1}, \tag{31}$$

$$\frac{\sigma_1}{\sigma_0} = \left(\frac{\gamma - 1}{\gamma + 1} + \frac{2}{\gamma + 1}\frac{1}{M^2}\right)^{-1}, \tag{32}$$

where $M = (U - u_0)/w_0$ denotes the Mach Number of the shock under consideration, U being the velocity with which the shock propagates in space so that $U - u_0$ is the velocity through the medium in front of it.

It should also be noted that, across the shock front, the quantities μ and x are continuous.

The foregoing equations disclose that if M increases (i.e., if either U increases or w_0 diminishes), the shock strength π_1/π_0 may increase beyond any limit, while the ratio σ_1/σ_0 possesses a finite upper bound. In neutral gas, the latter is clearly $(\gamma + 1)/(\gamma - 1)$ which, in air ($\gamma = 1.4$) is approximately 6, and for monatomic gas ($\gamma = 5/3$), diminishes to 4. At high temperatures, when the dissociation and ionization must be taken into account, the situation becomes more complicated[9] and the density jump across the shock may increase up to ten[10] which is, however, still of the same order of magnitude.

These facts have an important bearing on the interpretation of certain salient features of the Novae, whose effective photospheres we propose to identify with shock fronts at the head of expanding gas flow. Perhaps the most revealing pertinent feature is the fact that, as the radius of the effective surface of a Nova expands a hundred to a thousand times (a process, which we can follow by the integration of the light curves), the spectroscopic evidence indicates that the effective temperature declines as a rule

TABLE 2

WAVE FRONTS		PATH LINE	
(A)	$dx = (u \pm w)d\tau$	(D)	$dx = ud\tau$ (double)
(B)	$\frac{d\pi}{\gamma\pi} \pm \frac{du}{w} = -\left(\frac{2u}{x} \pm \frac{2\mu}{wx^2}\right)d\tau$	(E)	$d\pi - w^2 d\sigma = 0$
(C)	$d\mu = \pm\sigma x^2 w d\tau$	(F)	$d\mu = 0$ (double)

but very little—a fact which could scarcely be understood if the distribution of pressure in the expanding field of flow were to be continuous. The presence of a shock wave enables us, however, to account logically for this apparent paradox: for as the shock sweeps outwards, an integration of the light curve discloses that its velocity remains approximately constant, but the temperature of the tenuous gas in front of it diminishes rapidly with increasing distance from the center. As a result, the Mach Number M of the shock grows in proportion; and it is—we suggest—this gradual increase in shock strength, caused by the falling temperature in front, which maintains the observed temperature behind the shock in the course of its expansion, and by keeping the pressure sufficiently high it renders the shock front opaque. Thus, while an initial explosion—single or repeated—of as yet unknown origin (possibly a sudden release of nuclear energy, or gravitational collapse of a critical shell) in the interior of a Nova must be responsible for the onset of its outburst, it is the conversion of the kinetic energy into heat through the medium of shock waves which very probably gives rise to the effective photospheres of the Novae as observed in the early stages of expansion. A more complete discussion of the astrophysical implications of

our suggestion and a comparison with the observations is, however, outside of the scope of the present note and is being postponed for a subsequent publication.

Whereas the outer boundary of the field of flow caused by a sufficiently intense explosion will, we suggest, be of the nature of an expanding shock front, the inner boundary may or may not be a shock wave, depending on the energy balance of the phenomenon. For consider the limiting case of a blast wave caused by an instantaneous central explosion; the inner boundary of the corresponding flow will be a sphere of a radius x_1 equal to the instantaneous position of a particle that was originally at the center. If, by definition, all the energy which has produced the wave motion was released at $\tau = 0$, there will be no longer any energy exchange for $\tau > 0$ between the gas flow and the region interior to x_1. In consequence, our shock wave conditions reduce to

$$\begin{aligned} u(x_1) &= U \\ \pi(x_1) &= \pi_i \\ \sigma(x_1) &= 0 \end{aligned}$$

where π_i is an arbitrary constant. A sphere of radius x_1 thus becomes a surface of contact discontinuity, which moves with the gas at a velocity $u(x_1)$ and separates two regions of possibly different densities and temperatures, though the pressure remains the same on either side.

Mathematically speaking, the streamline of a particle which was originally at the center is evidently $\mu = 0$, and it has been shown in the preceding section that its direction is characteristic. Hence, for pure blast waves, the inner boundary itself proves to be a characteristic, a fact which should greatly facilitate the computations. Physically speaking, the core interior to x_1 can be visualized as a region of vanishing density, in which a finite pressure may be maintained by a very high temperature. It is obvious, however, that the period of time during which such a contact surface can actually exist is rather severely limited by the effects of heat conduction, which we have so far ignored in our equations. Nevertheless, the conduction of heat in stellar interiors seems such that our approximation may retain its usefulness during the first few hours, or even days, following the main explosion.

5. *Analytical Treatment of Boundary Conditions.*—In applying the boundary conditions in the method of stepwise integration, the conditions on a contact surface can be applied without much further investigation, since it is a characteristic itself. However, the conditions on the shock must be treated separately.

Let us first consider what kind of relations hold along the shock front By equations (30) to (32), all the three quantities π_1, σ_1 and u_1 behind the shock can be determined from known values π_0, σ_0, u_0 of these quantities in

front, together with the strength of the shock, which may be described, e.g., by the Mach Number M of the wave speed relative to the gas in front. The speed of propagation U of the shock wave in space is similarly determined. The quantities μ and x are continuous across the shock, and it is easy to see that along the shock front

$$dx = Ud\tau \tag{33}$$

$$d\mu = \sigma_0 x^2 v_0 d\tau = \sigma_1 x^2 v_1 d\tau, \tag{34}$$

where $v_0 = U - u_0$ and $v_1 = U - u_1$ are, respectively, the wave speeds relative to the gas in front and behind. These differential relations, together with the shock relations (30) to (32) can be used for the purpose of stepwise integration.

However, to develop a complete analogy between the shock front and the characteristic, one would like to obtain differential relations connecting the variables π_1, σ_1 and u_1 along the shock front. These may be found as follows. We know that

$$\begin{aligned} \pi_1 &= \pi_1(M, \pi_0, \sigma_0, u_0), \\ \sigma_1 &= \sigma_1(M, \pi_0, \sigma_0, u_0), \\ u_1 &= u_1(M, \pi_0, \sigma_0, u_0), \end{aligned}$$

while π_0, σ_0, u_0 are known functions of (x, τ) or (μ, τ). Thus

$$d\pi_1 = \frac{\partial \pi_1}{\partial M} dM + \left(\frac{\partial \pi_1}{\partial \pi_0} d\pi_0 + \frac{\partial \pi_1}{\partial \sigma_0} d\sigma_0 + \frac{\partial \pi_1}{\partial u_0} \right) du_0,$$

where $d\pi_0$, $d\sigma_0$, du_0 can be expressed in terms of $d\tau$ by relations of the type

$$d\pi_0 = \left(\frac{\partial \pi_0}{\partial x} \right)_\tau dx + \left(\frac{\partial \pi_0}{\partial \tau} \right)_x d\tau = \left\{ \left(\frac{\partial \pi_0}{\partial \tau} \right)_x + \left(\frac{\partial \pi_0}{\partial x} \right)_\tau U \right\} d\tau$$

or

$$d\pi_0 = \left\{ \left(\frac{\partial \pi_0}{\partial \tau} \right)_\mu + \sigma_0 x^2 v_0 \left(\frac{\partial \pi_0}{\partial \mu} \right)_\tau \right\} d\tau.$$

Thus,

$$d\pi_1 = \frac{\partial \pi_1}{\partial M} dM + A d\tau \tag{35}$$

$$d\sigma_1 = \frac{\partial \sigma_1}{\partial M} dM + B d\tau \tag{36}$$

$$du_1 = \frac{\partial \mu_1}{\partial M} dM + C d\tau \tag{37}$$

where A, B, C are known in terms of conditions at the shock front, and are independent of the coordinate system chosen. From (35) to (37), one may obtain, for example, the following differential relation connecting $d\pi_1$ and du_1:

$$\frac{\partial u_1}{\partial M}\, d\pi_1 - \frac{\partial \pi_1}{\partial M}\, du_1 = \left(A \frac{\partial u_1}{\partial M} - C \frac{\partial \pi_1}{\partial M} \right) d\tau. \tag{38}$$

Equations (33), (34) and (38) then correspond to those in table 2, and may be used in a similar manner. Again, form (38) is not dependent on the choice of x or μ as the independent variable.

The above merely gives the principle of the derivation. In practice, it is possible [by eliminating M between (30) and (31)] to get a simple algebraic relation between π_1 and u_1 containing π_0, σ_0, u_0. Thus, (38) can be obtained directly. Also, the quantities U, A, B, C may be most conveniently expressed in terms of both π_0, σ_0, u_0 and π_1, σ_1, u_1. These expressions will be given elsewhere.

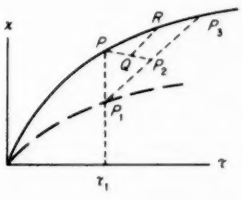

FIGURE 2

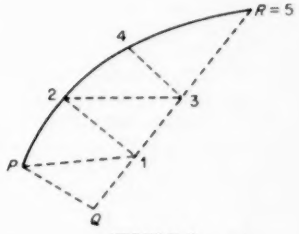

FIGURE 3

To fix our ideas on the process of stepwise integration involving a shock, let us consider it in some detail. Suppose the solution is known up to $\tau = \tau_1$; then it is also known in the triangle PP_1P_2 (Fig. 2). Furthermore, this part of the solution can be calculated by stepwise integration as explained in Section 3. The solution is also known in the triangular region PP_2P_3 bounded by the shock PP_3 and the two characteristics PP_2 and P_2P_3. To see the geometrical relations shown in the diagram, one should bear in mind that for an outgoing wave, $u_0 + w_0 < U < u_1 + w_1$.

To carry out the numerical integration, one may take a segment PQ of PP_2 so small that PR and QR are also small enough to justify the approximation of differentials by finite differences. One may then determine the values of x and τ for the point R and then the values of u_1 and π_1 by using the differential relations (33), (34), (38) developed above. The other two quantities μ and σ are determined by the continuity of μ across the shock and the shock relations (30) to (32). Iteration may be carried out in the usual manner.

Modification of the above procedure to get better accuracy might be desirable in certain cases. One may, for example, complete the integration of the triangular region PQR in the manner indicated in the following diagram (fig. 3), where $(P, 1)$ is the path line through P, and the characteristic $(1, 2)$ is drawn so that $< P1Q = < P12$, $(2, 3)$ is again a path line, etc.

Numerical integrations of the system (18) to (21) along the above-described lines, which should follow the propagation of spherical blast waves in the interior of stellar models approximating the structure of white subdwarfs, are now in progress at the Massachusetts Institute of Technology, and their results will be reported in subsequent communications.

* An investigation supported by Contract N5-ori-07843 with the Office of Naval Research.

[1] Rosseland, *Ap. J.*, **104**, 324 (1946) (a note in which certain analogies with the behavior of incompressive fluids are drawn). Schatzman, *Compt. rend.* (Paris), **222,** 722 (1946); *Ann. d'Astroph.*, **9,** 199 (1946), **12,** 281 (1949). Kopal, Symposium on Shock Wave Phenomena, U. S. Naval Ordnance Lab. Rept. No. 1135, 1950, pp. 1–11.

[2] Kopal, *Ap. J.*, **113,** 193, 496 (1951).

[3] Cf. Strömgren, "Colloque International d'Astrophysique," Actualités Scientifiques et Industrielles, No. 896, Hermann et Cie, Paris, 1941, pp. 159–161; Kopal and Shapley (unpublished).

[4] The literature concerning this equation is extensive and, to some extent, controversial. Of principal investigations we may mention Jeans, *M. N.*, **85,** 917 (1925), **86,** 328, 444 (1926); Rosseland, *Ap. J.*, **63,** 342 (1926); Vogt, *A. N.*, **232,** 1 (1928); Milne, *M. N.*, **89,** 518 (1929), *Quart. J. Math.* (Oxford), **1,** 1 (1930); *Handb. d. Ap.*, Bd. III (Zweite Hälfte), Berlin (1930), pp. 804–811; Thomas, *Quart. J. Math.* (Oxford), **1,** 239 (1930); Woltjer, *B. A. N.*, **7,** 127 (1933).

[5] Cf. Schatzman, *Bull. Acad. Roy. de Belgique* (Classe des Sciences, 5th Série), **34,** 828 (1948).

[6] Courant and Friedrichs, *Supersonic Flow and Shock Waves*, p. 70 ff. Note also the possibility mentioned there of actually reducing the problem to one involving only two dependent variables. This is theoretically possible here (using the Lagrangian treatment). Practically, however, the method used below is at least just as convenient.

[7] Lin, *Quart. Appl. Math.*, **7,** 443–445 (1950).

[8] Courant and Friedrichs, *Ibid.*, p. 149, eqs. (67.10) and (67.07).

[9] Cf. e.g., Bethe and Teller, "Deviations from Thermal Equilibrium in Shock Waves," Ballistic Research Lab. Rept. No. X-117, Aberdeen, Md.

[10] Fuchs, Kynch and Peierls, "The Equation of State of Air at High Temperature," H. M. Stationery Office MS No. 61, London, 1944.

THE TRANSFER OF RADIOACTIVE PHOSPHORUS FROM PARENTAL TO PROGENY PHAGE

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Introduction.—Reproduction is perhaps the most basic and characteristic feature of life. From the chemical point of view it is also the most obscure feature: atoms do not reproduce. When a living organism reproduces, there are now two atoms in the system for each one of the parent system. The additional atoms, of course, have not been "generated" by reproduction of the parent's atoms, but have been assimilated from the environment. Although the two progeny organisms may be biologically identical we should consider that their atoms can be classified into two classes: parental atoms and assimilated atoms. How are these atoms distributed between the two progeny organisms? Is one of the progeny all parental, the other all assimilated, or each half and half? Or perhaps both assimilated and the parental atoms dissimilated and passed into the environment? Are there specific macromolecular structures (genes?) that are preserved and passed on intact to the progeny? To answer questions of this kind we must be able to distinguish between parental and assimilated atoms and, in principle, this can be accomplished by the use of tracers.

Bacteriophages are especially suited for such studies because the division of their life cycles into inter- and intra-cellular phases enables one to label the virus particles without labeling the cellular environment within which they multiply. This was first done by Putnam and Kozloff[1] using phage T6r$^+$ active on *Escherichia coli* strain B, labeled with P^{32}. These authors infected unlabeled bacteria in unlabeled medium with labeled phage and found that 30% of the label of the infecting particles was transmitted to material characterized as phage by means of differential centrifugation. Similar results have been obtained with the phages T2r$^+$ and T4r$^+$ by Lesley, French and Graham.[2] This finding gives rise to numerous questions regarding the nature of this transmitted phosphorus: (1) Its origin: does it come from a specific part of the infecting particle; (2) its mode of transmission: is it transmitted in the form of large blocks of DNA or after complete breakdown and reassimilation; (3) its distribution: is it in one particle of the progeny, or distributed over all of them; (4) its location within the progeny particles: in specific parts of them, or distributed at random?

In consideration of recent work showing a genetic system in phage, Putnam and Kozloff suggested that the 30% transfer might be due to a bipartite structure of the phage, a genetic and a non-genetic part, and that

only the genetic part is transmitted. The P^{32} which is lost could then be considered as belonging to dispensable portions of the phage which are always lost during reproduction. At a phage meeting in Cold Spring Harbor in August, 1950, Dr. S. S. Cohen suggested that this hypothesis might be tested by allowing the progeny phage particles, which contain 30% of the parental P^{32}, to go through another cycle of reproduction in unlabeled bacteria. This experiment will be referred to as the "second generation experiment." If the notion of Putnam and Kozloff is correct, the "second generation experiment" should give 100% transmission, since the parental particles of this experiment would be specifically labeled in the transmissible part.

We have carried out experiments of this type by growing phage in labeled bacteria in labeled medium. These phages were allowed to infect unlabeled bacteria to produce progeny phage containing 30% of the radioactivity of the parental phage (first generation experiment). When the progeny phage were used to infect unlabeled bacteria, it was found that again 30% of the label is transferred to the progeny (second generation experiment).

This finding says, in essence, that the progeny particles are similar to the parental particle with regard to the localization of the phosphorus label. Since the parental particles of the first generation experiment were labeled uniformly throughout the particles, the same must be said about the progeny particles. Our experiment thus rules out Putnam and Kozloff's hypothesis—and answers the fourth of the questions listed above. It answers this fourth question only with respect to phosphorus. A different answer might be obtained with a label like sulphur, that would label specifically the protein moiety of the phage. The other three questions listed above remain unanswered by our experiment.

Material and Methods.—$T2r^+$ bacteriophage and the mutant B/1 of *Escherichia coli* strain B were used; the latter was chosen because contamination of plates with T1 bacteriophage occurs in our laboratory. The broth used in the transfer experiments is a watery meat extract to which is added 1% peptone, 0.02% Tween 80 and 0.5% NaCl; the pH is adjusted to 7.4. The latent period of $T2r^+$ in this medium is 22 minutes at 37° C. For preparation of radioactive stocks, a synthetic medium, **g**, of low phosphate concentration was used. It has the following composition:

NaCl 5 g.; NH_4Cl 1 g.; $MgSO_4(7\ H_2O)$ 0.1 g.; $CaCl_2$ 0.1 g.; $FeSO_4$ $(7\ H_2O)$ 0.01 g.; *dl*-tryptophane 0.01 g.; glycerol 2 g.; gelatin 0.01 g.; Tween 80 0.2 g.; casamino acids (Difco) 1 g.; KH_2PO_4 0.005 g.; Na_2HPO_4 0.01 g. To these substances were added 1000 cc. of distilled water and the pH adjusted to 7.1 with NaOH.

Considerable amounts of P were introduced with the casamino acids, bringing the total phosphate concentration up to 15.5 γ/cc. All experi-

ments were conducted at 37° C. The general methodology used was similar to that described by Adams.[3] All centrifugations were done in a Servall Angle centrifuge at 10° C. In some experiments ultra-violet irradiated T2r$^+$ was used to inhibit lysis or to lyse from without; a heavy dose of irradiation giving about 50 hits per particle[4] was administered just before the phage was to be used. Measurements of radioactivity were made on 0.15-cc. liquid samples by means of an end-window G.M.-tube. Under these conditions 1 μC of P^{32} gave 4×10^5 counts/min., an efficiency of 20%.

Experimental.—Preparation of P^{32} Labeled Bacteriophage: T2r$^+$ was labeled by growth on B/1 in *g* medium containing 5 μC P^{32} per cc. After incubating several days at 37° C. the phage was purified by differential low- and high-speed centrifugation. The phage was first resuspended in 0.05% NaCl since at this low salt concentration complexes consisting of phage plus bacterial debris dissociate so that the debris can be removed by centrifugation.[5] The phage was finally resuspended in *g* medium. For subsequent experiments it was important to make sure that, apart from phage, the preparation contained no P^{32}-labeled material which would adsorb on bacteria; to remove any such material heat killed B/2 was added, left in for 30 minutes and then removed by low-speed centrifugation. Less than 10% of the P^{32} of the preparation were removed in this way; it should be noted, however, that dead phage particles are not removed by this treatment. The final preparation contained 1.5×10^{11} particles/cc. with a radioactivity of 5×10^5 counts/min./cc. In phage prepared similarly by Lesley, French and Graham over 95% of the P^{32} was located in DNA.[6] Our stock adsorbed well on exponentially growing B/1, consistently giving over 97% adsorption in 4 minutes when the B/1 concentration was 5×10^8 cells/cc. This phage was used to infect bacteria.

Experiment I. Transfer of P^{32} During the First Generation of Phage Growth.—Exponentially growing bacteria from an aerated culture in unlabeled broth were concentrated in the centrifuge at 10° C. and resuspended in unlabeled broth at 37° C. to give a final concentration of 5×10^8 cells per cc. Labeled T2r$^+$ was then added at a ratio of 2 phage particles per bacterium. Under these conditions approximately 90% of the phage was adsorbed in 1 minute. Two minutes after infection, the culture was chilled, and centrifuged at 5000 g. for 4 minutes. The supernatant containing unadsorbed radioactive material was discarded and the pellet resuspended in broth at 37° C. to give a final concentration of infected cells of approximately 10^8/cc. A phage assay was then made to determine the number of infected cells, and aeration was started. Five minutes after resuspension, approximately 5 particles per bacterium of unlabeled, ultra-violet (UV) irradiated T2r$^+$ were added to inhibit lysis uniformly in the whole culture.[7] On incubation overnight the culture cleared and was centrifuged twice at

5000 g. for 5 minutes to remove bacterial debris and then at 12,000 g. for 1 hour to sediment the phage. The various pellets were resuspended in broth and assayed for P^{32} and viable phage. When assaying for phage, intermediate dilutions were made into distilled water and were kept for 10 hours to remove bacterial debris which temporarily inactivates phage by blocking its ability to adsorb.[5] The number of active particles often rises by a factor of 2 to 3 after distilled water treatment.

Table 1 shows the results of this experiment in which 37% of the radioactivity was found in the high-speed pellet. This pellet also contained the large majority of the progeny phage. To determine what fraction of the radioactivity of the high-speed pellet was actually in progeny phage, anti-T2 rabbit serum, which had been absorbed previously with a great amount of live B/1 to remove possible antibacterial antibodies, was added to the resuspended pellet at a final dilution of 1 to 10. The mixture was incubated at 45° C. for 2 hours and left at 4° C. overnight. The precipitate of phage and antibody which formed was collected by low-speed centrifuga-

TABLE 1

DISTRIBUTION OF RADIOACTIVITY AFTER INFECTION WITH P^{32} LABELED T2r$^+$

Bacterial concentration = 1.05×10^8/cc. Adsorbed phage particles/bacterium = 2.1.

Burst size = 465.

MATERIAL	PHAGE TITERS/CC.	RADIOACTIVITY IN 20 CC. COUNTS/MINUTE		%
Lysate	4.9×10^{10}	14,140		...
Low-speed pellets	0.10×10^{10}	940		6.2
High-speed pellet	4.2×10^{10}	5,590	15,080	37.0
High-speed supernatant	0.15×10^{10}	8,550		56.8

tion and was found to contain over 90% of the radioactivity originally present in the high-speed pellet. It is thus highly probable that the P^{32} in the high-speed pellet was largely present as phage material.

The possibility remains, however, that the radioactivity is not associated with the progeny phage but with slightly altered infecting particles that sediment at high speed. The question might be decided by examining the infected bacteria midway in the latent period just before the first infective particles begin to appear.[8] If after artificial lysis at this time the radioactivity is in sedimentable fragments of the infecting phages we might expect to be able to isolate them. On the contrary if this radioactivity is associated with progeny particles, that are not yet large enough to sediment, then we might not find much radioactivity in the high-speed pellet.

Experiment II. Distribution of Parental P^{32} Before the First Progeny Particle Appears in an Infected Bacteria.—Bacteria in the logarithmic phase of growth were concentrated to 5×10^8 cells/cc. and infected with an average of 6 labeled T2r$^+$ particles per bacterium. Two minutes after infec-

tion, the culture was chilled and centrifuged at 5000 g. for 4 minutes to remove unadsorbed radioactive material. The pellet was resuspended in broth at 37° C. at a bacterial concentration of 10^8 cell per cc. Aeration was started and an assay made of the number of infected bacteria. The cooling and centrifugation retards the progress of phage growth by about 8 minutes.

Thirteen minutes after infection 5 UV-inactivated T2r$^+$ particles per bacterium were added to inhibit lysis. At 19 minutes, corresponding to about time 11 minutes in the experiments of Doermann, the culture was divided into two tubes, A and B. To tube A was added sufficient KCN to make a final concentration of $^1/_{1000}$ M and approximately 2000 UV-treated T2r$^+$ particles per bacterium. By this means, the infected bacteria are broken open and any newly formed particles released into the medium.[8] Tube B was aerated until 42 minutes when it was similarly treated. The large excess of UV-treated T2r$^+$ served both to break open the bacteria and to saturate the bacterial surface.[9] Readsorption of the newly formed particles onto unlysed bacteria or bacterial debris is thus effectively prevented.[5]

TABLE 2

DISTRIBUTION OF RADIOACTIVITY AFTER PREMATURE LYSIS OF BACTERIA INFECTED WITH LABELLED T2r$^+$

Bacterial concentration = 1.12×10^8/cc. Adsorbed phage particles/bacterium = 6.7.

TUBE	TIME OF ADDITION OF KCN + UV T2r$^+$	PROGENY PHAGE PER BACTERIUM	P^{32} DISTRIBUTION, %		
			LOW-SPEED PELLET	HIGH-SPEED PELLET	HIGH-SPEED SUPERNATANT
A	19 minutes	0	12	1.6	86.5
B	42 minutes	248	13	29	58

The cultures were then incubated for 2 hours, by which time complete clearing (lysis) had occurred. They were then centrifuged twice at 5000 g. for 5 minutes to remove bacterial debris and finally at 12,000 g. for 1 hour to sediment phage. The excess of UV-treated T2r$^+$ served as carrier for any possible compounds which might be sedimented at high speed in tube A. The various pellets were resuspended in broth and assayed for P^{32} and active phage.

In table 2 the results are presented. It can be seen that the bacteria in tube A were broken open before any progeny particles were present, while in tube B approximately 250 particles per cell were present. The amount of P^{32} in the low-speed pellets was approximately the same in both tubes, 12% of the input. In striking contrast to this similarity between the low-speed pellets, the high-speed pellets from tubes A and B contained 1.6 and 29% of the input P^{32}, respectively. The P^{32} in the high-speed pellet taken

after lysis thus cannot be due to slightly altered infecting particles but represents the incorporation of the P^{32} into the progeny phage. Furthermore, this experiment can be considered as additional evidence of the breakdown of the infecting particle, first postulated by Doermann on the basis of premature lysis experiments.[8] In experiments of this kind virtually all the P^{32} left in the supernatant after high-speed centrifugation can be precipitated by addition of 5% trichloroacetic acid.

The above transfer experiment has been repeated with ratios of adsorbed phage to bacteria ranging from 1 to 6. The transfer of radioactivity to the progeny particles ranged from 20 to 40%, but was not correlated with the number of adsorbed particles per bacterium. There furthermore does not seem to be a noticeable correlation between the burst size and the degree of transfer. While the burst sizes varied from 150 to 600, the transfer percentage in most experiments ranged between 28 and 33%.

TABLE 3

DISTRIBUTION OF RADIOACTIVITY AFTER INFECTION WITH T2r$^+$ LABELED BY ONE GENERATION OF GROWTH IN NON-LABELED MEDIUM AND BACTERIA

Bacterial concentration = 1.32×10^8/cc. Adsorbed phage particles/bacterium = 5.2.

Burst size = 316.

MATERIAL	PHAGE TITERS/CC.	RADIOACTIVITY IN 150 CC. COUNTS/MINUTE	RADIOACTIVITY IN 150 CC. %
Lysate	4.17×10^{10}	390	100
Low-speed pellets	0.08×10^{10}	57.5	14.7
High-speed pellet	3.84×10^{10}	115	30
High-speed supernatant	0.38×10^{10}	[a]	

[a] Due to large volume, this could not be counted.

It has been shown by French, Lesley, Graham and Van Rooyen that late adsorbed phage is broken down rapidly.[10] The transfer percentage found in experiments like our No. I, would be too low if some of the progeny had been lost in this way through readsorption unto unlysed bacteria; however, in experiments like our No. II, when readsorption has been prevented, we find similar transfer values and therefore conclude that in our experiments readsorption is not an important factor.[5]

Experiment III. Transfer of P^{32} from Labeled Phage Which Has Gone Through One Cycle of Reproduction in Unlabeled Bacteria: The radioactive phage obtained in the high-speed pellet of experiment I was used to infect non-labeled bacteria in non-labeled broth. Bacteria in the logarithmic phase of growth and at a concentration of 1.3×10^8 cells/cc. were infected with an average of 5 phage particles per bacterium. Following an adsorption period of 5 minutes, the infected bacteria were centrifuged at 5000 g. for 5 minutes to remove unadsorbed materials and were resuspended in the original volume of broth. Aeration was started and 5 minutes later, 5 UV-

treated, unlabeled T2r$^+$ particles per bacterium were added to inhibit lysis. Following overnight incubation, the phage was isolated by differential centrifugation and assays made of the radioactivity and phage in the various fractions.

The results, shown in table 3, indicate a transfer to the progeny of 32%—that is a value similar to that obtained upon reproduction of phage labeled by growth on labeled bacteria in labeled medium.

Conclusion and Summary.—Bacteriophage T2r$^+$ has been labeled with P^{32} by growth in labeled bacteria in labeled medium. This phage has been carried through two successive cycles of reproduction in unlabeled bacteria. In both cycles it was found that the progeny phage had received about 30% of the P^{32} of the infecting phage particles. It is concluded that the phosphorus which is transferred to the progeny is not located, after transfer, in specific parts of the DNA of some or all of the particles of the progeny.

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*A SINGLE-CELL ANALYSIS OF THE TRANSMISSION OF ENZYME-FORMING CAPACITY IN YEAST**

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Introduction.—Winge and Roberts[1] reported that certain yeast strains can be distinguished by the fact that they take 5 to 6 days longer than normal varieties to adapt to galactose. They named this phenomenon "long-term adaptation" and showed that the character was determined by a single recessive allele (g_s) which segregated normally in crosses to the fast adapting wild type, bearing the dominant gene (G).

The characterization of phenotype in such instances involves extensive growth in the adaptive medium. As a consequence, the phenomenon was open to the interpretation that it involved mutation from a negative allele to the dominant genotype and subsequent selection of this mutant. Mundkur and Lindegren[2,3] offer genetic data obtained with their own stocks which suggest that this may indeed be the case in certain instances of long-term adaptation.

This question was analyzed by Spiegelman, Sussman and Pinska[4] using a strain closely related to the one employed by Winge and Roberts.[1] The results of this analysis made it evident that mutation and selection, in the accepted sense in which these terms are employed, cannot explain the phenomenon of "long-term adaptation" in this material. The data indicated that contact with substrate induces in a small proportion of the cells (about 1 in a thousand) a heritable modification resulting in capacity to form the adaptive enzyme. This capacity is maintained and transmitted indefinitely in the presence of galactose. Growth in the absence of the adaptive substrate results in reversion to the negative phenotype.

Analysis of the reversion kinetics revealed that it was a mass phenomenon involving over 90% of the cells present at the time of its occurrence. The progeny of positive cells retain and transmit the positive phenotype for the first 6 to 7 generations. At the end of this period, quite abruptly, the absolute number of positives in the culture ceases to increase and negative cells make their appearance. The generation at which the "burst" of negative cells occurs was found to be independent of the growth rate maintained during the course of the reversion.

These results led to the conclusion that long-term adaptation involves a cytoplasmically transmitted enzyme-forming system which can increase in the presence of substrate. The reversion to the original phenotype in the absence of substrate suggests that little or no formation of the enzyme-synthesizing system occurs under these circumstances and consequently it is diluted during the period of growth. A point is finally reached where cells are produced possessing either none or an insufficient number of the elements responsible for enzyme formation.

Since yeast cells reproduce by budding, it is in principle possible to distinguish between parent and daughter cell. Under such circumstances it becomes experimentally relevant to raise certain questions highly pertinent to an understanding of the mechanism underlying the reversion. Thus, one would like to know whether the original positive cells can revert to the negative phenotype or whether this phenomenon is confined solely to the progeny derived from them. It would also be of interest to know the quantitative relations which obtain between the probability of obtaining a negative daughter cell and the number of divisions which have occurred in a reverting medium.

Data on these and related problems can provide information pertinent to such questions as the number of elements initially present and the minimal number required for the manifestation of the positive phenotype.

The most direct experimental analysis of such problems would involve isolation and characterization of the progeny as they appear serially during the division of a positive cell in the absence of substrate. This, in principle, is the method routinely employed in the study of inheritance phenomena in protozoa and which has in the hands of Sonneborn[5] and Preer[6] led in recent years to such remarkable advances in our understanding of the transmission mechanics of cytoplasmic factors in these forms. More recently Ephrussi and Hottinguer[7] have successfully applied this methodology to the appearance of respiratory mutants ("petites") in yeast. A direct experimental approach of this kind can be used in any instance in which the frequency of the appearance of identifiably different daughter cells is sufficiently high. The analysis of the reversion phenomenon indicates that it satisfies this criterion.

It is the purpose of the present paper to present the results of such progeny-analysis experiments. The data obtained support the conclusions of the cytoplasmic nature of the phenomenon drawn from the previously reported[4] study of a reverting culture. They indicate further that the elements responsible for enzyme formation are transmitted in a random manner and that there are relatively few of them (between 100 and 200) in a fully adapted positive cell.

Methods.—(*A*) *Strain:* The yeast strain C_1d is the same as that used in our previous[6] studies and is a representative of *Saccharomyces chevalieri*. (*B*) *Media:* Carbohydrate-free medium was made by adding the following to 1 liter of H_2O; $(NH_4)_2SO_4$, 6 g; bacto-peptone, 5 g.; $MgSO_4$, 1 g.; KH_2PO_4, 2 g.; difco-yeast extract 2.5 g. Agar (20 g.) was added to the above when a solid medium was required. Galactose medium was made by adding 20 g. of purified galactose (PG) to a liter of the above medium. Merck's galactose was purified by recrystallization from 70% alcohol. The reversion medium for the present experiments contained 0.1% glucose. (*C*) *Test Plates:* As was demonstrated previously[4] Eosine Methylene Blue-purified galactose (EMB-PG) test plates can be employed to distinguish between positive and negative cells. (*D*) *Cultures:* All the cultures employed in the present experiments were derived from 18 to 24 hr. PG-broth cultures inoculated with positive clones and incubated at 30° C. in standing tubes. (*E*) *Cell Isolation and Phenotype Scoring:* (1) Single cells were isolated by means of a deFonbrune manipulator and transferred to separate drops of 0.1% dextrose broth. These were held on a cover slip fixed to a Winge-type operating chamber. To insure that cells selected were capable of effective budding, only those showing incipient bud formation were isolated. (2) The isolated cells were incubated at 30° C. and examined at

intervals. When the buds formed by such cells were of sufficient size (about $^3/_4$ that of the mother cell) they were separated from the mother cell and transferred by means of a micropipette to a drop of 2% galactose-0.075% glucose medium held on another cover slip which was then placed on a Van Tieghem chamber. Each bud so isolated was incubated in a separate chamber. (3) After 24 hrs. at 30 °, these drops, containing between 500 and 2000 cells, were transferred and spread on EMB-PG test plates and read after 3 days of incubation. No ambiguity in scoring the phenotype was encountered since positive cells give rise in the droplets to clones composed of cells all of which yield positive clones on the test plate. Negative cells, on the other hand, give rise to clones in which the vast majority of the cells are negative.

The inclusion of the 0.075% glucose in the test droplet was found necessary in order to obtain good recovery of the isolated buds. This is probably due to the fact that the cells deadapt enzymatically in the reversion medium and lose enzyme more rapidly than they lose enzyme-forming sys-

TABLE 1

PHENOTYPE OF BUDS PRODUCED BY POSITIVE CELLS DIVIDING IN THE ABSENCE OF SUBSTRATE

Plus sign indicates that the clone derived from the single cell was positive on test plate; negative sign indicates the clone was negative. A zero indicates that the corresponding bud failed to develop and a blank space, that bud isolation was terminated prior to the corresponding generation.

GENERATIONS									MOTHER CELL
1	2	3	4	5	6	7	8	9	
+	+	0	+	+	+	−	−	0	−
+	+	+	+	+	−	+	−	−	−
+	+	+	+	+	+	−			+
+	+	+	+	+	+	+			−

tem. The small amount of glucose presumably provides sufficient energy for growth and readaptation for those cells potentially capable of doing so.

Results.—*(A) Nature of the Pedigrees:* Using the above procedure about 40 relatively complete pedigrees were obtained. A few representative ones illustrating some of the points at issue are given in table 1. By virtue of their origin, and as confirmed by control platings of the populations from which they were derived, all of the mother cells in these as well as in the other pedigrees to be considered started out as positive cells. The results illustrated answer two of the questions raised in the introductory paragraphs. It is evident that in addition to yielding negative daughter cells after a certain number of divisions, a positive cell can itself revert to the negative phenotype. Indeed, if a sufficient number of divisions are permitted, such a reversion is almost invariable. Thus, of 9 mother cells isolated after the formation of 9 buds, 7 were of the negative phenotype and 2 were positive; and of 6 isolated after the formation of 10 buds, all were of

the negative phenotype. As is further illustrated by the second pedigree in table 1, alternation of the phenotype of the daughter cells can occur in successive generations.

It would clearly be a matter of some difficulty to explain such progeny pedigrees on a mutational basis. The data suggest that after a certain number of divisions have occurred, the number of elements available for the distribution is such that the two cells produced as a result of a division cannot both be positive. In some instances the daughter cell receives a sufficient number, leaving the mother cells with fewer than are required, and in other cases the reverse is observed. A situation of this nature would be expected if either of the following two conditions obtained at the time of the cell division: (*a*) the number of elements being distributed is large and the minimal number required for the positive phenotype is large; or (*b*) the number of elements being distributed is small and the minimum number required is also small.

A decision between these alternatives as well as other aspects of the transmission mechanism can be made with the aid of the quantitative data on the proportion of positives found at each generation.

(*B*) *Quantitative Aspects of the Reversion to the Negative Phenotype:* The data obtained in the course of the single-cell analyses are summarized in table 2 in terms of the generation and phenotype of each daughter cell isolated. The method used makes these data amenable to relatively direct quantitative interpretation since the origin and generation of each cell scored is defined with certainty. It has previously[4] been noted that the rate of cell division does not influence the generation at which the reversion phenomenon sets in. The number of elements responsible for the positive phenotype, and which are being diluted during cell division, may therefore to a first approximation be regarded as remaining at a relatively constant level during the reversion. Under these circumstances a Poisson analysis should adequately describe the observations.

With a constant number of elements, the proportion of positives to be expected at any given generation in the course of a single-cell analysis will depend on the following three parameters: p_0, the number of elements initially present; $1/d$, the fraction of the parental elements which pass into the daughter cell; and finally on ν, the minimal number required to yield the positive phenotype.

At the time of cell division, the bud receives $1/d$th of the number of elements present and the mother cell retains $(1-1/d)$ of this amount. It follows that the average number of elements, p_n, in an nth generation daughter cell will be given by

$$p_n = \frac{p_0}{d}\left(1 - \frac{1}{d}\right)^{n-1}. \qquad (1)$$

On the other hand, the average number of elements, $p'_{n'}$ remaining in a mother cell after it has produced n' daughter cells is given by

$$p'_{n'} = p_0 \left(1 - \frac{1}{d}\right)^{n'}. \tag{2}$$

The expected proportion of positive cells, P_n, among the nth generation buds will then be given by

$$P_n = \sum_{j=\nu}^{\infty} \frac{(p_n)^j}{j!} e^{-p_n}, \tag{3}$$

where ν is, as above, the minimal number of elements required for a cell to be positive under the conditions of the test.

With the aid of equation (3), theoretical curves can be constructed relating the proportion of positive buds with the generation for different values of the three parameters. The major effect of increasing p_0 is to increase the number of generations before the appearance of negative cells. Since even large variations in p_0 do not detectably alter the shape of the descending part of the curve, this parameter cannot play a critical role in the comparison of theoretical and experimental curves. In practice p_0 is estimated from the generation of the 50% point (1:1 ratio of positives to negatives) after the values of the other parameters have been determined.

Both ν and d markedly affect the form of the curve and they do so in opposing directions. Increasing ν increases the rate of descent whereas larger values of d tend to make the curve fall more slowly. It is evident that different combinations of these two parameters can yield similar curves. An independent estimation of at least one of these parameters is therefore necessary. The nature of the bud analysis experiment permits a determination of d independent of the absolute values of either ν or p_0. The reasonable assumption may be made that the minimum number of particles required for a cell to be positive does not depend on whether it happens to be a mother or a daughter cell. It follows that the average number of particles in a group of mother cells will be equal to that in a group of buds whenever the per cent of positive cells in the two groups are equal. Consequently, if after the production of the n'th generation bud, the proportion of positives among the mother cells is equal to the proportion of positives among the nth generation buds, we may set p_n and $p'_{n'}$ of equations (1) and (2) equal to each other. We thus obtain

$$\left(1 - \frac{1}{d}\right)^{n'} = \frac{1}{d}\left(1 - \frac{1}{d}\right)^{n}. \tag{4}$$

Since n' and n are experimentally determinable, an estimation of d is possible. In the course of the present experiments 17 mother cells were re-

covered and scored after yielding 8 buds. Of these, 6 or 35% were positive. As may be seen from table 2, this compares with 33% for 8th generation buds. Of 14 mother cells isolated after the 7th generation, 10 or 71% were positive, which compares with 65% for 7th generation buds.

Evidently, n' closely approximates n, and from equation (4), it may be calculated that d must be in the neighborhood of 2. This method is actually a relatively sensitive one for determining d. Thus, for $d = 3$, the difference $(n' - n)$, instead of being close to zero, would have to attain a value of 1.7 generations and for $d = 4$; a value of 3.8 generation would be required for $(n' - n)$. We may, therefore, with some assurance place the value of d between 2 and 3, and in the comparison of theory with experiment disregard those distributions which involve values of d greater than 3.

Using equation (3) and p_0 equal to 100, theoretical distribution curves are plotted in figure 1 for different combinations of d equal to 2 and 3 and ν equal to 1 and 2. On the same graph are placed the experimentally ob-

TABLE 2

SUMMARY OF PHENOTYPES OF DAUGHTER CELLS PRODUCED BY POSITIVES DURING DIVISION IN A REVERSION MEDIUM

GENERATION	POSITIVES	NEGATIVES	TOTAL	% POSITIVES
2	34	0	34	100
3	37	0	37	100
4	27	0	27	100
5	27	1	28	96.5
6	26	6	32	81.5
7	22	12	34	65.0
8	9	18	27	33.3
9	3	10	13	23.0

served distribution of positives in the daughter cells as described in table 2. It is evident that the observations are very well described by the distribution predicted from the assumption that $\nu = 1$; $d = 2$; i.e., that 1 particle is necessary and sufficient for the exhibition of the positive phenotype, and that the probability of any particular particle passing into the bud is $1/2$.

Though the agreement is close, it does not establish beyond doubt that $\nu = 1$; $d = 2$ is the only possible pair of values. Thus ($\nu = 3$; $d = 3$) yields a 50% point of 7.3 generations as compared with 7.2 for the ($\nu = 1$; $d = 2$) combination, and relatively good agreement at the other points. The estimation of d made above makes a value close to 3 unlikely but not impossible. There exists, however, another experimental device which can help decide between these alternatives. It involves a comparison between the proportion of positives obtained at each generation by the single-cell and populational analysis methods.

These two methods can yield similar quantitative results only when d is

close to 2. The extent of the divergence for other values of d may be estimated from the relation deduced in the following paragraphs.

The proportion of positives to be expected at each generation during a populational analysis of a reversion may be derived if it is recalled that in a growing culture the generations are measured in terms of the number of doublings. Let $\bar{n}$ designate the number of generations estimated in this manner. Any given sample removed from the culture will be a mixture of cells derived from different generations. Direct observation indicates that the division frequency does not vary significantly from one cell generation to the next. Under these circumstances, the proportion of ith generation cells to be expected at the $\bar{n}$th doubling of the culture, will be given by the binomial coefficients ${}_{\bar{n}}C_i$.

Following the argument used previously, the average number of particles in the parental cells at the $\bar{n}$th generation will be given by $p_0(1 - 1/d)^{\bar{n}}$. Each succeeding ith generation representative in the $\bar{n}$th doubling will have $[1/(d-1)]^i$th of the corresponding parental number. Consequently the average number of particles possessed by an ith generation cell in the $\bar{n}$th generation of the culture is given by

$$p_{\bar{n},\,i} = p_0 \left(1 - \frac{1}{d}\right)^{\bar{n}} \left(\frac{1}{d-1}\right)^{i}. \tag{5}$$

The proportion of positives among such ith generation cells is

$$P_{\bar{n},\,i} = \sum_{j=\nu}^{\infty} \frac{(p_{\bar{n},\,i})^j}{j!} e^{-p_{\bar{n},\,i}}. \tag{6}$$

Summing $P_{\bar{n},\,i}$ over all the i generation types existent in each $\bar{n}$th generation yields $P_{\bar{n}}$, the corresponding total proportion of positives in the culture at the $\bar{n}$th doubling. We thus obtain

$$P_{\bar{n}} = \sum_{j=\nu}^{\infty} \sum_{i=0}^{\bar{n}} {}_{\bar{n}}C_i \frac{(p_{\bar{n},\,i})^j}{j!} e^{-p_{\bar{n},\,i}} \tag{7}$$

as the relation between the proportion of positives and the generation in a reversion followed by the populational analysis method. It is evident from equation (5), and the fact that the sum of the binomial coefficients is unity, that for d equal to 2, $P_{\bar{n}}$ equals $P_{\bar{n}}$ of equation (3).

Relation (7) can be used to estimate the adequacy of (ν, d) combinations which cannot be distinguished easily by the use of equation (3). The comparison of predicted with experimental values is most conveniently effected at the point where $P_{\bar{n}}$ is 0.5. This point can be easily determined with accuracy since it is the intersection of the positive and negative curves. Using equation (7), and a p_0 value of 100, it can be shown graphically that for $\nu = 3$; $d = 3$, $P_{\bar{n}}$ will equal 0.5 at an $\bar{n}$ of 5.1. On the other hand, for

$\nu = 1$; $d = 2$, $P_{\bar{n}}$ assumes the value of 0.5 at $n = 7.2$. The difference of 2.1 generations between the two predicted values can be distinguished experimentally without difficulty.

In 21 population reversion experiments it was found that the 0.5 point fell at an $\bar{n}$ of 6.9 ± 0.4 generations. The poor agreement ($p < 0.01$) between the experimental and the 5.1 value predicted by $\nu = 3$; $d = 3$ makes this combination very unlikely. On the other hand, the good correspondence between the generation of the 0.5 point obtained by the single-cell and populational analysis methods adds further support for a value of d close to 2. If one accepts this estimate of d, the curves and experimental points of figure 1, as well as the good agreement between the observed and

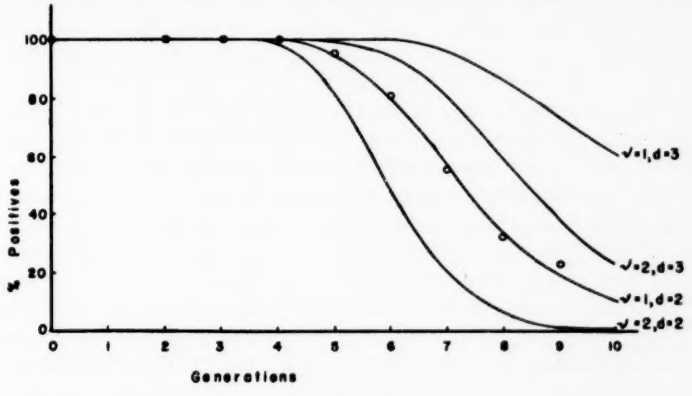

FIGURE 1

Theoretical curves relating the proportions of positive among progeny with generation for various combinations of ν, the minimum number required for the positive phenotype, and $1/d$, the probability that any particular particle is transmitted to the daughter cell. The circles represent the experimental observations summarized in table 2.

predicted values of the 0.5 point in the populational reversion experiment, lead to the conclusion that ν must be in the neighborhood of 1.

Discussion.—The relative directness with which relevant data are obtained in the single-progeny analysis experiments and the quantitative correspondence with theory make it difficult to avoid the conclusion that a particulate distribution phenomenon is being observed. Little can as yet be said about the nature of these particles. The available data do, however, suggest the following conclusions concerning them: (*a*) their presence in adequate numbers is necessary for the possession and transmission of galactozymase forming capacity; (*b*) they are randomly distributed between parent and daughter cells; (*c*) they do not increase in number in the

absence of substrate; (*d*) enzymatically adapted cells contain between 100 and 200 particles; (*e*) if present, they can be maintained indefinitely at a constant level during logarithmic growth of the cells in the presence of substrate.

The fact that a certain small proportion of the progeny derived from a negative cell invariably possesses the capacity to generate particles on being brought into contact with substrate suggests the existence of a mechanism for replacing lost particles. One interpretation of the replenishing mechanism which may be offered is that it is genic. Thus it may be that a cell carrying the dominant gene (*G*) possesses a mechanism for producing particles in the absence of substrate of such efficiency as to insure that each cell formed contains one or more of such particles. However, a cell carrying the recessive allele (g_s) possesses a generating mechanism of such inefficiency that only one out of about 1000 divisions results in a cell with one or more particles.

It is important to emphasize that the inefficiency of g_s-genotype cells may reside not in the actual process of particle production but rather in their inability to retain them due to the existence in such cells of an active destructive mechanism when substrate is absent. It is also evident that the origin of the responsible particles need not be nuclear. They could be derived by a mutational process from other particulate elements present in non-adaptable cells of the g_s genotype. The available data do not as yet permit a decision among the various possible alternatives.

The quantitative interpretation given to the parameters of distribution in the experiments reported here can, in principle, be confirmed by other experimentally independent methods. This is particularly true for the values of the minimal number, ν, and the initial number p_0. One obvious method for arriving at such independent determinations follows from the fact that if $\nu = 1$, the absolute number of positives in the course of a reversion should approach asymptotically a limiting value equal to (p_0N_0) where N_0 is the number of positive cells in the inoculum. For higher values of ν, the absolute number of positives would reach a maximum somewhere near the 50% point and then should fall to zero. Such experiments would not only test the value of ν but also permit an estimation of p_0. However, accurate estimation of the absolute number of positives in the region of interest becomes technically difficult for two reasons: (*a*) the positives comprise a progressively smaller proportion of the population, making their accurate count difficult; (*b*) as has been previously demonstrated,[4] the presence of large numbers of negatives has a deleterious effect on positives. Methods are now being worked out to overcome these difficulties. The preliminary experiments indicate that conditions can be achieved in which the absolute number of positives approaches a plateau yielding a p_0 corresponding to the number calculated by the theory presented here.

The results reported here on the transmission of the capacity to form galactozymase parallel in many respects the investigations of Ephrussi[3] and his coworkers on the inheritance of cytochrome oxidase in yeast. The data of these workers also suggest the existence of randomly distributed particulate elements responsible for enzyme formation. In this case, however, control of the number of elements per cell is not as easily achieved by variation of substrate in the medium and cells lacking particles are produced by treatment with euflavine. It is of interest to note that it has been possible to demonstrate in the course of the present investigation that the particulate elements involved in cytochrome oxidase production are distinct from those responsible for the formation of the galactozymase system. Exposure of galactose adapted cells to euflavine[9] yields typical cytochrome oxidase deficient mutants which, however, possess the capacity to form galactozymase and can transmit this capacity to progeny indefinitely in the presence of substrate. Such "petite" mutants revert in the usual manner to negative "petites" when grown in the absence of galactose.

Summary.—The reversion from the positive to negative phenotype in a yeast strain exhibiting long-term adaptation to galactose has been analyzed by serially isolating and characterizing single-progeny cells produced by positives during division in the absence of substrate. The results may be summarized as follows: (*a*) Under the conditions employed, positive cells begin to produce negative progeny at the 5th division; (*b*) the proportion of negative progeny increases with each succeeding generation; (*c*) beyond the sixth generation positive and negative progeny can be alternately produced in successive generations by the same mother cell; (*d*) a parent cell can revert to the negative phenotype while producing a positive daughter cell; (*e*) at any given generation, the proportion of reversions among mother cells is the same as that among the daughter cells.

Analysis of the quantitative relations between the proportion of positives indicates that particulate elements responsible for enzyme formation are being randomly distributed between mother and daughter cells. The data indicate that the initial number in an adapted cell is about 100, the minimal number required for the positive phenotype is in the neighborhood of 1 and the probability of any particle passing into the daughter cell is close to $^1/_2$.

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† Standard Brands Post-Doctoral Fellow in Yeast Genetics.

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THE CLASS-NUMBER OF REAL QUADRATIC FIELDS

BY N. C. ANKENY, E. ARTIN, AND S. CHOWLA

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Let $h = h(d)$ denote the class-number of the real quadratic field $F = R(\sqrt{d})$ of fundamental discriminant d; let $\epsilon = \frac{t + u\sqrt{d}}{2} > 1$ be its fundamental unit. Further let $\chi(\nu)$ denote the character belonging to F, and $d = pm$ where p is an odd prime, and m is an integer. Further $[x]$ denotes the greatest integer contained in x, and $\left(\frac{n}{p}\right)$ is Legendre's symbol of quadratic residuacity.

Then we have the following results.

THEOREM 1. *If $p > 3$, $m > 1$,*

$$-2h\frac{u}{t} \equiv \sum_{0 < \nu < d} \frac{\chi(\nu)}{m\nu}\left[\frac{\nu}{p}\right] \pmod{p}.$$

In the case $p = 3$ there is an additional factor $(1 + m)$ on the left side of this equation.

A special case of Theorem 4 is

THEOREM 2. *If $d = p \equiv 5(8)$, we have*

$$+ 4\frac{u}{t}h \equiv -\sum_{1}^{p/4} \frac{1}{n}\left(\frac{n}{p}\right) \pmod{p}.$$

Write

$$\chi(\nu) = \left(\frac{\nu}{p}\right)X(\nu),$$

so that $X(\nu)$ is a real primitive character (mod m). Then we have

THEOREM 3. *If*

$$\sum_{-1}^{\infty} \frac{C_n x^n}{n!} = \frac{\sum_{t=1}^{m} X(t)e^{tx}}{e^{mx} - 1}.$$

Then if $p > m$, $p \neq 3$,

$$-2h\frac{u}{t} \equiv C_{(p-3)/2} \pmod{p}.$$

Finally let A denote the product of all the quadratic residues of p lying between 0 and p, B the product of the quadratic non-residues of p lying between 0 and p. Then

THEOREM 4. *If* $d = p \equiv 1(4)$, *then*

$$2h\frac{u}{t} \equiv \frac{A + B}{p} \pmod{p}.$$

ON THE BASIS THEOREM FOR FINITE ABELIAN GROUPS (SECOND NOTE)

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1. The author has recently published a simple proof of the fundamental theorem mentioned in the title,[1] wherein the analogy provided by the determination of a basis for a finitely generated vector space is followed.[2] In the present note we give another quite simple proof, based on a certain minimum condition.

Still a third proof will appear in a future issue of these PROCEEDINGS.

Recently, R. Rado has also given an interesting proof of the basis theorem,[3] using a method different from any of the author's. His treatment is based on a readily established lemma equivalent to the following: if $c_1, c_2, \ldots, c_m$ are any integers not all zero, then a determinant D with integer elements exists which includes the c's as first row and whose value is the g.c.d. of the c's.

2. In virtue of the well-known expression of any abelian group, of order

$$g = p_1^{\alpha_1} p_2^{\alpha_2} \ldots p_k^{\alpha_k}$$

($p_1, p_2, \ldots, p_k$ distinct primes), as direct product of abelian groups of the respective orders $p_1^{\alpha_1}, p_2^{\alpha_2}, \ldots, p_k^{\alpha_k}$, we may restrict ourselves to the case of prime-power order.

Let then G be an abelian group of order $g = p^{\alpha}$. We shall use the additive

notation for the composition of elements, so that the identical element will be denoted by 0.

By a *system of generators* of G we shall mean a sequence of elements of G: $\theta_1, \theta_2, \ldots, \theta_m$ (distinct or not) such that every element θ of G is expressible in the form

$$\theta = a_1\theta_1 + a_2\theta_2 + \ldots + a_m\theta_m, \tag{1}$$

the a's being integers. A *basis* of G:

$$B = (\theta_1, \theta_2, \ldots, \theta_m), \tag{2}$$

is, by definition, a system of generators which does not include the zero, and whose elements are independent in the following sense: the relation

$$c_1\theta_1 + c_2\theta_2 + \ldots + c_m\theta_m = 0 \tag{3}$$

shall imply

$$c_i\theta_i = 0 \qquad \text{for } i = 1, 2, \ldots, m, \tag{4}$$

or, what is equivalent,

$$c_i \equiv 0 \pmod{\pi_i} \qquad (i = 1, 2, \ldots, m). \tag{5}$$

Here π_i denotes the period of θ_i, so that

$$\pi_i\theta_i = 0 \qquad (i = 1, 2, \ldots, m). \tag{6}$$

Necessarily, each π_i, as divisor of $g = p^\alpha$, is a power of p:

$$\pi_i = p^{r_i}. \tag{7}$$

(We may say that the θ's shall satisfy no linear relation not deducible linearly from (6). An equivalent requirement is that the representation (1) of an arbitrary element in terms of a basis be essentially unique, meaning to within a linear combination of (6). It is also evident that the elements of a basis must be distinct, since $\theta_1 = \theta_2$, or $\theta_1 - \theta_2 = 0$, would imply $\theta_1 = 0, \theta_2 = 0$, contrary to the requirement that a basis shall not contain the zero element.)

We define the *index* of a system of generators to mean the sum[4] of the periods of its elements:

$$I = \pi_1 + \pi_2 + \ldots + \pi_m. \tag{8}$$

Certainly, at least one system of generators of G exists, e.g., the one composed of all the elements of G taken in any order. Since the index is always a positive integer, there follows the existence of at least one system of generators of *minimum* index.

Let the system B in formula (2) have this minimum property; we say that B must then be a basis.

3. *Proof:* First, one may remark that certainly none of the θ's in B can be zero; otherwise the index of B could be decreased by omission of such elements without depriving B of its character as system of generators—this is obvious by reference to (1).

To establish the independence of B, suppose, on the contrary, that we had the relation (3) with

$$c_j \not\equiv 0 \pmod{\pi_j},$$

j a specific one of the indices $1, 2, \ldots, m$. Then

$$c_j = q_j\pi_j + d_j, \text{ with } 0 < d_j < \pi_j, \tag{9}$$

q_j, d_j integers.

By use of (6) and relations like (9) we can replace each coefficient c_i in (3) by its remainder mod π_i, so getting

$$d_1\theta_1 + d_2\theta_2 + \ldots + d_j\theta_j + \ldots + d_m\theta_m = 0, \tag{10}$$

where

$$0 \leqq d_i < \pi_i \qquad (i = 1, 2, \ldots, m), \tag{11}$$

and where certainly $d_j > 0$, by (9).

Accordingly, there is a highest power of p, $p^\lambda(\lambda \geqq 0)$, dividing all the d's, so that

$$d_i = p^\lambda e_i \qquad (i = 1, 2. \ldots, m) \tag{12}$$

and at least one e_i is *not* divisible by p. To fix the ideas, say that p does not divide e_3.

Then (10) may be rewritten in the form

$$p^\lambda(e_1\theta_1 + e_2\theta_2 + e_3\theta_3 + \ldots + e_m\theta_m) = 0.$$

Defining now

$$\theta_3' = e_1\theta_1 + e_2\theta_2 + e_3\theta_3 + \ldots + e_m\theta_m, \tag{13}$$

we have

$$p^\lambda\theta_3' = 0;$$

therefore the period π_3' of θ_3' divides p^λ.

In summary, π_3' divides p^λ, which divides d_3 (by (12)), which is less than π_3 (by (11)); hence

$$\pi_3' < \pi_3. \tag{14}$$

Let us now combine (13) with the following particular equation of (6):

$$0 = \pi_3\theta_3. \tag{15}$$

π_3 is a power of p (by (7)), while e_3 is not divisible by p; thus e_3 and π_3 are relatively prime, and therefore integers x, y exist such that

$$xe_3 + y\pi_3 = 1.$$

Accordingly, by combining (13) and (15) linearly with the respective multipliers x, y, we get

$$x\theta_3' = xe_1\theta_1 + xe_2\theta_2 + \theta_3 + xe_4\theta_4 + xe_5\theta_5 + \ldots + xe_m\theta_m,$$

whence

$$\theta_3 = -xe_1\theta_1 - xe_2\theta_2 + x\theta_3' - xe_4\theta_4 - xe_5\theta_5 - \ldots - xe_m\theta_m.$$

By substituting this in (1), we see then that

$$B' = (\theta_1, \theta_2, \theta_3', \theta_4, \theta_5, \ldots, \theta_m)$$

is also a system of generators for G. But the index of B' is

$$I' = \pi_1 + \pi_2 + \pi_3' + \pi_4 + \pi_5 + \ldots + \pi_m;$$

therefore, by subtraction of (8),

$$I' - I = \pi_3' - \pi_3 < 0,$$

by (14), or $I' < I$.

Thus B does *not* have the minimal index postulated, and this contradiction completes our proof that B is indeed a basis.

[1] These PROCEEDINGS, **37**, 359–362 (June, 1951). Abstract in *Science*, **113**, 481 (April, 1951).

This proof, as well as the present one, has been in the author's notes since 1946.

Correction. In the PROCEEDINGS paper, p. 360, line following formula (3), read $i > j$ instead of $i < j$.

[2] For proofs in the literature, along quite different lines, see the algebraic treatises of Weber, H. (vol. 2), Fricke, R., Hasse, H., and also Pontrjagin, L., *Topological Groups*, Princeton University Press, 1940.

[3] *Journal of the London Mathematical Society*, **26** (part 1), 74–75 (1951).

[4] We could also use the product, but the basis so obtained might include any number of zero elements. On the other hand, minimum value of the product of the periods would be a necessary, as well as sufficient, condition for a basis.

In fact, we could employ as index any function $\varphi(\pi_1, \pi_2, \ldots, \pi_m)$ of the periods that is strictly monotone increasing in each argument, such as the sum of the nth powers, n a positive integer.

FOURIER ANALYSIS AND SYMMETRIC SPACES

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We shall consider in this note some applications of the generalized Peter-Weyl-Plancherel formula to certain special cases, where it is possible to obtain better and more precise results than in the general case of arbitrary locally compact unimodular (separable) topological groups. For this purpose we make the following definition.

DEFINITION 1. *Let G be a locally compact group and K a compact subgroup of G. Suppose there exists a Haar-measure preserving antiautomorphism σ of G and subset S of G satisfying the following conditions:*

(i) Every element g of G can be written uniquely and continuously in the form[1]

$$g = sk \text{ with } s \in S \text{ and } k \in K$$

(ii) $\sigma(s) = s$ and $\sigma(k) = k^{-1}$ for all $s \in S$ and $k \in K$.

Under these conditions G will be called a "Group with a Symmetry σ with respect to the Compact Subgroup K" or more briefly a "Group with a Symmetry."

Examples of groups satisfying these conditions are well known. For instance we may let G equal the group of all (real or complex) $n \times n$ matrices of determinant $\neq 0$ (or $= 1$) and K the unitary subgroup. With S equal to the set of positive definite Hermitean matrices in G and $\sigma(g)$ = complex conjugate transpose of g, the above conditions are easily verified. This example can be generalized by taking for G any complex semisimple Lie group and K a maximal compact subgroup; also many real semisimple Lie groups satisfy the above conditions (i) and (ii). An entirely different example is that of a semidirect product of a compact group K by a commutative locally compact group.

DEFINITION 2. *If G is a group with a symmetry with respect to a compact subgroup K then we call the homogeneous space G/K of (left-) cosets sK a Symmetric (homogeneous) space.*

This definition is an adaptation of Elie Cartan's symmetric Riemannian spaces[2] to the needs of generalized Fourier analysis; but it should be noted that we do not make any assumptions that G/K be a Riemannian space or even a differentiable manifold. Thus if $K = (1)$ we obtain as a special case all commutative locally compact groups. On the other hand if G is a semisimple Lie group we obtain a large class (but not all) of Elie Cartan's

symmetric Riemannian spaces such as for instance Siegel's "Symplectic Geometry" [3] as a special case of definition 2 above.

The Haar-measure on G determines an invariant measure on G/K, by means of which we form the Hilbert space $L_2(G/K)$ of equivalence classes of square integrable complex valued functions defined on G/K. If f is an element of $L_2(G/K)$, it can be identified with an element $f(g)$ of $L_2(G)$ satisfying $f(gk) = f(g)$ for all $k \in K$, and we can define a unitary operator $U(\mathfrak{g})$ acting on $L_2(G/K)$ for every $\mathfrak{g} \in G$ by

$$[U(\mathfrak{g})f](g) = f(\mathfrak{g}^{-1}g). \tag{1}$$

We shall now indicate some properties of the unitary representation U: $g \to U(g)$ which will show that under our above assumptions (definition 2) the representation U of G behaves very much better than unitary representations do in general.

Let $a(g)$ and $b(g)$ be Haar-Lebesgue integrable functions on G satisfying $a(kgk') = a(g)$ and $b(kgk') = b(g)$ for all $k, k' \in K$ and $g \in G$. Then it follows from conditions (i) and (ii) above that [4] $\int a(g\mathfrak{g}^{-1})b(\mathfrak{g})\,d\mathfrak{g} = \int b(g\mathfrak{g}^{-1})a(\mathfrak{g})\,d\mathfrak{g}$ where $d\mathfrak{g}$ refers to Haar-measure on G. If we combine this with the lemma of reference 5 we may conclude:

LEMMA 1. *Let W' be the algebra of those bounded linear operators on $L_2(G/K)$ which commute with $U(g)$ for every $g \in G$. If G is a group with a symmetry with respect to the compact subgroup K, then W' is commutative.*

From this one obtains several important consequences: Let W denote the (weakly closed self-adjoint) ring of operators generated by the operators $U(g)$. Then *Lemma 1 implies that W is a ring of Type* I. In fact if we perform the direct integral decomposition

$$L_2(G/K) = \int_{\oplus} H_t \tag{2}$$

to which the commutative algebra W' belongs in the sense of reference 6 then W consists of all bounded operators which are decomposable under (2) in the sense of theorem V of reference 6. In particular it follows that von Neumann's central decomposition and the decomposition[7] into irreducible spaces are the same in our present special case (up to equivalence of direct integrals), namely, both equal to (2).

Another consequence of Lemma 1 is obtained if we use the generalization of the Frobenius reciprocity theorem outlined in reference 5. Let V_t: $g \to V_t(g)$ be the strongly continuous unitary representation of G obtained in the space H_t for almost every t in accordance with Theorem 1.1 of reference 8. Then we may conclude that *the subspace of those elements of H_t which are invariant under the operators $V_t(k)$ for all $k \in K$, is one-dimensional for almost every t.* The use of this will be one of the main steps in the argument outlined below

These results can now be applied to obtain a rather explicit expression for the generalized Peter-Weyl-Plancherel formula for the space $L_2(G/K)$ when G/K is a symmetric space in the sense of definition 2 above. Let $f(g) \in L_1(G) \cap L_2(G)$ and satisfy $f(gk) = f(g)$ for all $k \in K$; i.e., we assume $f \in L_1(G/K) \cap L_2(G/K)$. Then the operator $F(t) = \int f(g) V_t(g)\, dg$ is a bounded operator on H_t for almost every t and $F(t)$ is an element of $W(t)$, where $W(t)$ denotes the algebra obtained from W in the space H_t under the decomposition (2). *The above implies that $W(t)$ is for almost every t the ring of all bounded operators on H_t.* Hence if one applies §7 of reference 8 one can show[9] that $F(t)$ must be of the Hilbert-Schmidt class for almost every t and

$$\int_G |f(g)|^2\, dg = \int \mathrm{Tr}[F(t)F(t)^*]a(t)\, ds(t) \tag{3}$$

where Tr denotes the *ordinary* trace of a (finite or infinite dimensional) matrix, $ds(t)$ refers to the measure with which the direct integral (2) is formed and $a(t)$ is a certain real valued function (cf. part IV of reference 6 and §7 of reference 8).

It can now be shown that it is possible to introduce in the space H_t a complete orthonormal system $e_1(t), e_2(t), \ldots$ in a measurable manner (i.e., a measurable family in the sense of definition 2 of reference 6) such that $V_t(k)e_1(t) = e_1(t)$ and the (K-invariant) subspace of H_t spanned by $e_2(t), e_3(t), \ldots$ does not contain any non-zero elements invariant under $V_t(k)$ for all $k \in K$. Moreover the inner product

$$(F(t)e_m(t), e_n(t)) = 0 \text{ for } n \neq 1 \text{ and all } m = 1, 2, 3, \ldots \tag{4}$$

as follows from the equation $f(g) = f(gk)$ together with the above properties of $V_t(k)$. Hence if we put

$$\hat{f}_m(t) = (F(t)e_m(t), e_1(t)) = \int_G f(g) V_t(g)_{m_1}\, dg \text{ for almost all } t \tag{5}$$

where $V_t(g)_{m_1}$ denotes the inner product $(V_t(g)e_m(t), e_1(t))$ in the space H_t then we obtain from equation (3)

$$\int |\hat{f}(g)|^2\, dg = \int \sum_m |\hat{f}_m(t)|^2 a(t)\, ds(t). \tag{6}$$

From this it follows in a well-known manner that the mapping $f(g) \to \hat{f}_m(t)$ can be uniquely extended to a unitary mapping defined for all $L_2(G/K)$ provided the definition (5) of $\hat{f}_m(t)$ is replaced by l. i. m. $\int f(g) V_t(g)_{m_1}\, dg$, where l. i. m. has to be taken (as usual) along an ascending family of compact subsets of G whose union is G.

Now let $f'(g)$ and $f''(g)$ be any two functions $\in L_2(G)$ both invariant under right translations by all elements of K and assume that their convolution $f(g) = (f' * f'')(g)$ is again square integrable. Then one can deduce from the above for their generalized Peter-Weyl Fourier transforms (5)

$$\hat{f}_m(t) = \hat{f}_1'(t)\hat{f}_m''(t) \text{ for almost all } t. \tag{7}$$

Now suppose that $f(g) \in L_2(G)$ and satisfies $f(kgk') = f(g)$ for all $k, k' \in K$. Then the theory of the generalized Peter-Weyl Fourier analysis takes on an especially simple form for such functions $f(g)$, because $f(kgk') = f(g)$ can be shown to imply

$$\hat{f}_m(t) = 0 \text{ for all } m \neq 1 \text{ and almost all } t \tag{8}$$

Hence one obtains for the generalized Fourier transform (5) of such functions $f(g)$ a single function $\hat{f}(t)$ (and not a sequence of functions as above) given by

$$\hat{f}(t) = \text{l. i. m.} \int f(g) V_t(g)_{11} dg; \tag{9}$$

and (6) simplifies to

$$\int_G |f(g)|^2 \, dg = \int |\hat{f}(t)|^2 \, a(t) \, ds \, (t). \tag{10}$$

The function $V_t(g)_{11}$ is an elementary positive definite continuous function on G and the above implies $V_t(kgk')_{11} = V_t(g)_{11}$ for all k and $k' \in K$; thus the $V_t(g)_{11}$ are (elementary positive definite) *spherical* functions in the sense of Gelfand[4] (see also Harish-Chandra[10]). And according to our equations (9) and (10) *we have obtained a generalized Fourier expansion of the L_2-functions on G which are constant on the double cosets modulo K, in terms of certain* (not necessarily all) *elementary positive definite spherical functions.*

We conclude by mentioning that the above results hold under somewhat more general conditions than stated. For instance one can replace the above representation U of G by ind χ (as defined in references 11 and 5) where χ is any real valued continuous one-dimensional representation of K and still prove that the commuting algebra W' of ind χ is commutative when G is a group with a symmetry with respect to K. And it is the condition that W' be commutative under which the above methods and results apply. In cases where the commuting algebra of ind u (u now an arbitrary irreducible representation of K) is known to be a finite module over its center (such as when G is [the adjoint group of] a semisimple Lie group[12]) the above methods can be applied after some modification. The element $e_1(t)$ of the space H_t is then no longer unique (up to constant multiples) but has to be replaced by a certain finite dimensional subspace of H_t which transforms according to replicas of u under the operators $V_t(k)$, $k \in K$, as follows from the generalized Frobeinius reciprocity theorem.[5] Hence equation (4) and what follows after it have to be modified accordingly.

[1] This condition could be somewhat relaxed: It would be enough for our purposes to assume it for all g outside of some set of Haar measure zero. Also the condition that the mappings $g \to s$ and $g \to k$ be continuous is not quite necessary for what follows. However the interesting examples satisfy the conditions as stated.

[2] Cf. for instance Elie Cartan, *Verh. Intern. Math. Kongress*, 152–161 (1932) and the references given there.

[3] Siegel, C. L., "Symplectic Geometry," *Am. J. Math.*, **65**, 1–86 (1943).

[4] Gelfand, I., "Spherical Functions in Symmetric Riemann Spaces," *Dokladi Akad. Nauk* **70**, 5–8 (1950).

[5] Mautner, F. I., "A Generalization of the Frobenius Reciprocity Theorem," these PROCEEDINGS, **37**, 431–435 (1951).

[6] von Neumann, J., "On Rings of Operators. Reduction Theory," *Ann. Math.*, **50**, 401–485 (1949).

[7] Theorem 1.2 of Mautner, F. I., "Unitary Representations of Locally Compact Groups I," *Ibid.*, **51**, 1–25 (1950).

[8] Mautner, F. I., "Unitary Representations of Locally Compact Groups II," *Ibid.*, **52**, 528–556 (1951).

[9] Note that left and right invariant Haar measure are the same (up to multiplicative constants) under our assumptions (definition 1). But we assume of course that G is separable. Thus the results of §7 of reference 8 are applicable.

[10] Harish-Chandra, "Representations of Semisimple Lie Groups on a Banach Space," these PROCEEDINGS, **37**, 170–173 (1951).

[11] Mackey, G. W., "Imprimitivity for Representations of Locally Compact Groups I," *Ibid.*, **35**, 537–545 (1949).

[12] This follows from references 10 and 5.

*REMARKS ON THE LEBESGUE'S MEASURE EXTENSION DEVICE FOR FINITELY ADDITIVE BOOLEAN LATTICES**

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1. By a *Boolean tribe*† we shall understand a distributive and complemented lattice. If (A) is a tribe, its elements will be termed *somata*,‡ and their collection denoted by A. In a tribe, the partial ordering $\leq$ defines a specific notion of equality $a = b$ of somata defined by $a \leq b$, $b \leq a$. Operations $a + b$, $a \cdot b$, co a, $a - b = a \cdot \text{co } b$ are equality-invariant (co a means the complement of a). We shall use the symbols $\sum$, $\prod$ for infinite addition and multiplication, and we shall use Bourbaki—symbols $\bigcup$, $\bigcap$ when dealing with sets. Let (A), (A') be two tribes. We say that (A) *is a finitely genuine subtribe of* (A') and that (A') *is a finitely genuine extension of* (A), if the following (independent) conditions are satisfied for somata of (A): I, $a + b = c$ is equivalent to $a +' b =' c$; II, $a \cdot b = c$ is equivalent to $a \cdot' b =' c$; III, $\text{I} =' \text{I}'$; IV, $0 =' 0'$. It follows that $A \subseteq A'$, and that $a \leq b$ is equivalent to $a \leq' b$. If in addition to that $\sum_{n=1}^{\infty} a_n = b$ is equivalent to $\sum_{n=1}^{\prime\infty} a_n =' b$, we say that (A) is *a denumerably genuine subtribe of* (A').**

2. Let (A) (A') be two (finitely) additive tribes, and (A) be a finitely genuine subtribe of (A'). Let $\mu(a) \geq 0$ be a finitely additive, finite measure on (A), i.e., if $a \cdot b = 0$, then $\mu(a + b) = \mu(a) + \mu(b)$. We shall study the Lebesgue's measure-extension-device through denumerable coverings, aim-

ing at the extension of μ from (A) into (A'). The methods and arguments used here are available in the literature, hence we give only sketches of proofs. Nevertheless they were made, by the author, very cautiously and explicitly, because (1) there are two different kinds of equality to be taken into account, (2) infinite operations on somata are not so easy as for sets, for they are dependent on their total class, (3) infinite operations may be not always meaningful. Though, under these very general circumstances, rather complicated and paradoxical results could be expected in advance, the content of this paper will show that the corresponding theorems are simple and not diverge very much those in the classical measure theory for sets.

3. By *covering of* a' we shall understand any infinite sequence $\{a_n\}$ with $a_n \in A$ and with meaningful sum $\sum'_n a_n$ such that $a' \leq' \sum'_n a_n$. We define the *exterior measure of* a', $\mu'_e(a')$ as $\inf \sum_n \mu(a_n)$ taken for all coverings $\{a_n\}$ of a'. Obviously we can consider only those coverings for which $\sum_n \mu(a_n)$ converges, and even with disjoint a_n. We define $\mu'_i(a') = \mu'_e(\mathrm{I}) - \mu'_e(\mathrm{co}'\, a')$, *the interior measure of* a'. We have (1) $\mu'_e(0) = 0$, (2) if $a' \leq' b'$, then $\mu'_e(a') \leq \mu'_e(b')$, (3) if (A') is denumerably additive, and $a' =' \sum'^{\infty}_{n=1} a'_n$, then $\mu'_e(a') \leq \sum_{n=1}^{\infty} \mu_e(a'_n)$. If (A') is finitely additive, we have only $\mu'_e(a +' b') \leq \mu'_e(a') + \mu'_e(b')$, and (3) holds true for $a' \in A$. We have $0 \leq \mu'_i a' \leq \mu'_e(a')$ and $\mu'_e(a) \leq \mu(a)$. There are cases where $\mu'_e(a) < \mu(a)$, even when (A') is denumerably additive. E.g. Let A be the collection of all sets $a = R \cap \bigcup_i(\alpha_i \beta_i)$[††] where the union is finite, $0 \leq \alpha_i \leq 1$, $0 \leq \beta_i \leq 1$ and where R is the set of all rational numbers. If we define, on A, the relation as the set-inclusion, we get a finitely additive tribe (A). Let A' be the collection of all subsets of $(0, 1) \cap R$, with set-inclusion as ordering relation. Define $\mu(a) = \text{meas} \bigcup_i(\alpha_i, \beta_i)$. We have $\mu'_e(\mathrm{I}) = 0$, $\mu(\mathrm{I}) = 1$.

We say that a' is *measurable*, if $\mu'_i(a') = \mu'_i(a')$. Denote by L' the class of all measurable somata of (A'), and put $\mu^L(a') = \mu'_e(a')$ whenever $a' \in L'$.

4. In this N_0 and in N_0 5, 6, 7, we admit that (A') is *denumerably additive*. In the original Lebesgue's theory the proof of the denumerable additivity of measure is based on topological arguments which, in our general case, are not available. There are two non-topological theories of measure, one by Carathéodory[‡‡]), and another studied by the author on abstract tribes in particular case.[8] We start with the second which seems to be more natural and, if presented with details, takes but a little more space than the Carathéodory's theory.

We say that a', b' *do not differ by more than* $\epsilon > 0$, if there exists a covering $\{x_n\}$ of $(a' -' b') +' (b' -' a')$ such that $\sum_n \mu(x_n) \leq \epsilon$; *we shall write* $[a', b'; \epsilon]$. Define M' as the class of all somata a' of (A'), such that for every $\epsilon > 0$ there exists $a \in A$ with $[a', a; \epsilon]$. If $a' \in M'$, define a *sequence determining* a' as any sequence $\{a_n\}$ for which there exists $\{\epsilon_n\}$, $(\epsilon_n > 0,$

$\lim \epsilon_n = 0$) such that $[a', a_n; \epsilon_n]$. We easily prove that: $A \subset M'$; if $a' \in M'$, then $\text{co}'\, a' \in M'$; if $a_n' \in M'$ $(n = 1, 2, \ldots)$, then $\sum_{n=1}'^{\infty} a_n' \in M'$; if $[a', 0; \epsilon]$ for every $\epsilon > 0$, then $a' \in M'$, and if besides $b' \leq' a'$, then $b' \in M'$. Our aim is to prove that the classes L' and M' coincide, and we shall do this stepwise. Let us indicate these steps. First we prove that $A \subset L'$ and, by analogous reasoning, that if $a, b \in A$, $a \cdot b = 0$, then $\mu^L(a + b) = \mu^L(a) + \mu^L(b)$. If $a_1, a_2, \ldots a_n, \ldots \in A$, $a_i \cdot a_k = 0$, $(i \neq k)$, $a = \sum_{n=1}'^{\infty} a_n$, then $\mu^L(a) = \sum_{n=1}^{\infty} \mu^L(a_n)$. (This may be not true, if $a = \sum_{n=1}^{\infty} a_n$). We have $|\mu^L(a) - \mu^L(b)| \leq \mu^L[(a - b) + (b - a)]$. If $a' \in M'$, and $\{a_n\}$ is a sequence determining a', then $\lim \mu^L(a_n)$ exists. This limit does not depend on the choice of the sequence determining a'. We put $\mu^M(a') = \lim \mu^L(a_n)$. We have $\mu^L(a) = \mu^M(a)$. The following lemma gives the proof of the additivity of μ^M: If $a', b' \in M'$, $a' \cdot' b' = {}' 0'$, $[a, a'; \epsilon]$, $[b, b'; \epsilon]$, then there exists $a_1 \leq a$, $b_1 \leq b$ with $a_1 \cdot b_1 = 0$ and $[a', a_1; 2\epsilon]$, $[b', b_1; 2\epsilon]$. To prove the denumerable additivity of μ^M, we prove first that, if $a' \in M', a_n \in A$, $a' \leq' \sum_{n=1}'^{\infty} a_n$, then $\mu^M(a') \leq \sum_{n=1}^{\infty} \mu^L(a_n)$. Now we see that, if $\mu^M(a') = 0$ then $\mu_e(a') = 0$. If $a' \in A'$, then there exist $b' \in M'$, $c' \in M'$ such that $c' \leq' a' \leq' b'$, $\mu_i'(a') = \mu^M(c')$, $\mu_e'(a') = \mu^M(b')$. It follows that $L' \subset M'$ and that, if $a' \in L'$, then $\mu^L(a') = \mu^M(a')$. At this step the proof of $M' \subset L'$ is easy. Thus $M' \equiv L'$.

5. Let us consider the Carathéodory's device. The definitions and proofs of this theory can be taken from the quoted book by S. Saks[4] almost without any change. We recall briefly the basic definitions fitting an abstract denumerably additive tribe (B). A real valued function $\Gamma(x)$, defined for all $x \in B$, is said to be an outer measure on (B) if (1) $\Gamma(x) \geq 0$, (2) $\Gamma(0) = 0$, (3) if $x \leq y$, then $\Gamma(x) \leq \Gamma(y)$, (4) $\Gamma(\sum_{n=1}^{\infty} x_n) \leq \sum_{n=1}^{\infty} \Gamma(x_n)$. A soma x is said to be Γ-*measurable* if, for every $y \in B$, $\Gamma(y) = \Gamma(y \cdot x) + \Gamma(y \cdot \text{co}\, x)$. On the class of all Γ-measurable somata $\Gamma(x)$ is denumerably additive. Besides this class gives a denumerably additive tribe which is a denumerably genuine subtribe of (B).

6. The soma-function $\mu_e'(a')$ is a Carathéodory's outer measure on (A'). We can easily prove that $\mu_e'(a')$ is the biggest Carathéodory's outer measure Γ on (A'), such that $\Gamma(a) \leq \mu(a)$ for every $a \in A$. Denote by C' the class of all μ_e'-Carathéodory measurable somata of A'. We proceed to give the steps of a proof of the identity $L' \equiv C'$. By applying a method of Carathéodory[9] we prove that $A \subset C'$. It follows that C' contains the borelian extension of (A), provided that infinite operations are taken with respect to (A'). Given a', there exist $b_{ik} \in A$ and $c_{ik} \in A$ such that, $\sum_k' b_{1k} \geq' \sum_k' b_{2k} \geq' \ldots \geq' a'$, $\Pi_k' c_{1k} \leq' \leq \Pi_k' c_{2k} \leq' \ldots \leq' a'$, and such that, if we put $b' = {}' \Pi_i' \sum_k' b_{ik}$, $c' = {}' \sum_i' \Pi_k' c_{ik}$, we have $c' \leq' a' \leq' b'$, $\mu'(b') = \mu_e'(a')$, $\mu'(c') = \mu_i'(a')$. This gives $L' \subset C'$. The proof of the inclusion $C' \subset L'$ is easy. Thus we have proved that $L' \equiv C' \equiv M'$.

7. Under the above circumstances μ^L may be no extension of μ, because

it may happen that $\mu^L(a) < \mu(a)$.

THEOREM. *In order that* $\mu^L(a) = \mu(a)$ *for every* $a \in A$ *each of the following conditions is necessary and sufficient:*

I. *If* $\sum'^{\infty}_{n=1} a_n = 1$, *then* $\mu(1) \leq \sum^{\infty}_{n=1} \mu(a)$,

II. *If* $a_1 \geq a_2 \geq \ldots$ *with* $0 = \prod'^{\infty}_{n=1} a_n$, *then* $\mu(a_n) \to 0$,

III. *If* $a_1, a_2, \ldots$ *are disjoint and* $a = \sum'^{\infty}_{n=1} a_n$, *then* $\mu(a) = \sum^{\infty}_{n=1} \mu(a_n)$.

IV. $\mu'_e(a) = \mu(a)$ *for all* $a \in A$ (see references 8 and 10).

Notice that summations of somata are taken from (A'). In the general case we have the

THEOREM. *Every soma of* (A) *is measurable. The set* L' *of all measurable somata of* (A') *with ordering relation (on* L'*) defined as the restriction of* $\leq'$ *to* L', *makes up a denumerably additive tribe* (L') *which is a denumerably genuine subtribe of* (A'). (A) *is a finitely genuine subtribe of* (L'). *We have* $\mu^L(a) \leq \mu(a)$. $\mu^L(a)$ *is denumerably additive on* (L'). *The measure* $\mu^L(a)$ *defines on* (A'), *by means of coverings taken from* (L') *an exterior measure on* (A') *which coincides with* μ'_e. *The three methods (explained above) if applied to* (A), (A'), *and* μ *give the same results. The set* L' *has the property that if* $\mu^L(a') = 0$, $b' \leq' a'$ *then* $\mu^L(b') = 0$, *and so* $b' \in L'$.

8. This being settled, *let us drop the condition that* (A') *is denumerably additive.* The topic becomes more difficult and the results obtained are deeper than the preceding ones. To manage this general case we use a theorem by MacNeille,[6, 8] stating that for every tribe (A) there exists a denumerably additive (even completely additive) tribe (A^*) which is a denumerably genuine (even completely) extension of (A') through isomorphism. We may suppose that $A' \subset A^*$. By using the preceding results, we obtain on (A^*), and hence on (A') an exterior measure $\mu^*_e(a')$. Denote by A'_* the set of all somata of (A') which are μ^*-measurable. The ordering in (A') organizes A'_* into a finitely additive tribe (A'_*). Take another MacNeille extension (A^{**}) of (A'). The tribe (A^{**}) is a denumerably genuine extension of (A'_*). If we denote the corresponding exterior measure on (A') by $\mu^0_e(a')$, we prove that $\mu^0_e(a') = \mu^{**}_e(a')$, and that $\mu^0_e(a') \leq \mu^*_e(a')$. It follows that $\mu^{**}_e(a') \leq \mu^*_e(a')$. The two extensions (A^*), (A^{**}) of (A') being arbitrary, we deduce that $\mu^*_e(a')$ on (A') *is independent of the choice of MacNeille's extension.* We call $\mu^*_e(a')$ *weak exterior measure of* a' and speak of *weak measurability of somata of* (A'). *The tribe of all weakly measurable somata of* (A') *is a finitely genuine subtribe of* (A') *and contains* (A). Denote by (L^*) the tribe of all μ^*-measurable somata of (A^*) and consider the quotient tribe $(A^*)/J^*$ where J^* is the ideal of all somata of (A^*) whose μ^*-measure is zero.

9. Consider with MacNeille,[7] μ^*-fundamental sequences $\{a_n\}$ where $a_n \in A$, i.e., sequences for which $\mu^*(a_n - a_m) + \mu^*(a_m - a_n) \to 0$ as $n, m \to \infty$. We easily define somatic operations on them and a tribe $(A^\times)$, whose somata are these sequences. The class of all somata $\{a_n\}$ for which

$\mu^*(a_n) \to 0$ is an ideal $J^\times$. Now the following theorem holds true. $(A^*)/J^*$ *is isomorphic with* $(A^\times)/J^\times$. The tribe $(A^\times)/J^\times$ is completely additive and is a finitely genuine extension of the tribe of equivalence classes of (A) modulo the ideal composed of those somata a of (A) for which $\mu(a) = 0$ (see MacNeille[7].)

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† Boolean lattice, Boolean algebra, Boolean field. The term "tribe" is taken from René de Possel.[1]

‡ The term "soma" is taken from Carathéodory.[9]

** See the paper by the author, reference 2. This notion differs from that of a subset of A' close under finite and denumerable operations.

†† (α, β) is the set of all x for which $\alpha < x \leq \beta$.

‡‡ Reference 3 at end of paper; see, e.g., Saks's book.[4]

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ON THE CONSISTENCY OF "NEW FOUNDATIONS"

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The question of the consistency of the system of set theory in my "New Foundations,"[1] briefly NF, has long been mooted.[2, 3] The question takes on added interest with Wang's new revision[4] of the system of my *Mathematical Logic*;[5] for Wang has proved that this revision, which is to be used in the forthcoming third printing of *Mathematical Logic*, is consistent if NF is consistent. The purpose of the present note is to point out that NF is consistent if and only if the system of Whitehead and Russell's *Principia Mathematica*,[6] briefly PM, is consistent in a certain one of its natural versions.

PM may be conceived in a form in which the variables carry indices indicating logical type. Alternatively PM may be conceived under a convention which Whitehead and Russell called *typical ambiguity;* under this convention the indices are suppressed but still only those formulae are admitted as meaningful which are *stratified*, i.e., so constituted that type indices *could* be inserted everywhere without violating the theory of types. Another point on which we have a choice, in framing a version of PM, is the status of relations and relational predication: we may assume relational predication as primitive on a par with set-membership, and decree a sprawling annex to the theory of types to accommodate relations, as was done in effect by Whitehead and Russell; or alternatively we may, following Wiener,[7] reduce relations to certain sets of sets of sets (at the cost of giving up relations between things of different types). The four systems obtained by superimposing these two pairs of alternatives will be called PM1, PM2, PM3, and PM4, thus: PM1 uses type indices and admits the relational apparatus as primitive; PM2 uses typical ambiguity and admits the relational apparatus as primitive; PM3 uses type indices and assumes only the truth functions, quantification, and membership ('ϵ') as primitive; and PM4 uses typical ambiguity and assumes only the truth functions, quantification, and membership as primitive. All four systems are to be understood as not including the axiom of infinity or the axiom of choice.

PM2 is inconsistent, as Whitehead and Russell knew; cf. vol. 3, pp. 75, 80. On the other hand PM1 and PM3 are easily proved consistent.[8] What will concern us is PM4.

The axioms of PM4 are the *extensionality axiom:*

$$(x)(y)(z)[(w)(w \epsilon x \cdot \equiv \cdot w \epsilon y) \cdot x \epsilon z \cdot \supset \cdot y \epsilon z],$$

and the *abstraction axioms*, which comprise the closures (by universal quan-

tification of any free variables) of all stratified formulas $\ulcorner(\exists x)(y)(y \in x \cdot \equiv \phi)\urcorner$ such that ϕ has no free 'x'. The theorems of PM4 are all the stratified formulas *quantificationally implied* by axioms; i.e., all the stratified formulas ψ such that, for some conjunction χ of axioms, $\ulcorner\chi \supset \psi\urcorner$ becomes a valid schema of quantification theory (or first-order predicate calculus) when 'ϵ' is thought of as a schematic predicate letter.

Note that stratification of ψ assures that of $\ulcorner\chi \supset \psi\urcorner$; for the axioms in χ are stratified and devoid of free variables, and are hence freely combinable without breach of over-all stratification. It is still conceivable, however, that the proof of $\ulcorner\chi \supset \psi\urcorner$ in quantification theory might itself be irremediably unstratified; and in this event one might object that the proof cannot occur in PM4 (where stratification is essential to significance), and hence that ψ has no business among the theorems of PM4. But this objection will probably be withdrawn when one reflects that the curious phenomenon which we are imagining is a function of whatever particular systematization is chosen for quantification theory. Many systematizations of quantification theory are available, all of which are known to be complete and, in any ordinary sense, logically equivalent to one another; and it is only the trivial detail of our choice among these systems that decides whether certain stratified formulas, or certain different ones, or none at all, are dependent for their proofs upon excursions through unstratified formulas. Thus it seems more reasonable to specify the theorems of PM4 absolutely and independently of any particular systematization of quantification theory, by appeal to quantificational implication as in the preceding paragraph.

NF differs from PM4 in that stratification is not required for meaningfulness. The axioms of NF are the same as those of PM4, but the theorems of NF comprise *all* the formulas (stratified or not) which are quantificationally implied by axioms. Thus the *stratified* theorems of NF are exactly the theorems of PM4.

It follows that NF is consistent if and only if PM4 is consistent. For, if NF is inconsistent then every formula is a theorem of NF, and therefore both '$x \in y$' and its negation are theorems of PM4. Conversely, if PM4 is inconsistent then every stratified formula is a theorem of PM4, and therefore both '$x \in y$' and its negation are theorems of NF.

Incidentally the observation that every stratified theorem of NF is a theorem of PM4 has a certain interest over and above the proof of relative consistency. A so-called axiom of infinity, to the effect that Λ does not belong to all classes which contain 0 and are closed with respect to successor, has been discussed a good deal[3, 9] with regard to its demonstrability or indemonstrability in NF. Since it is a stratified formula, we can now say that its demonstrability in NF is only as likely as its demonstrability in PM4. More generally, given any stratified formula, the question of its demonstrability in NF or consistency with NF reduces to the same question

relative to PM4.

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*GROWTH RESPONSES OF OPPOSITE SIGN AMONG DIFFERENT NEURON TYPES EXPOSED TO THYROID HORMONE**

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Introduction.—The metamorphic changes transforming the tissues of the tadpole into those of the mature frog have been shown to be under the "control" of the thyroid hormone. That is, they fail to occur, if the hormone is absent. However, it is becoming increasingly clear that "control" does not mean the determination of the specific character of the ensuing changes, but refers merely to the reactivation and further sustenance of different chains of morphogenetic events, temporarily arrested in the larval stage, then continuing in each tissue reacting according to its own characteristic properties. Hormone action does not initiate heterogeneity in homogeneous tissues; it merely leads to the realization, including visualization, of latent differences based on pre-existing heterogeneity. The mosaic of terminal hormone effects is anticipated by a corresponding latent mosaic of differential susceptibility and response among the various reacting tissues (Weiss, 1924). In brief, the morphogenetic action of a hormone is not too unlike the action of the photographic developer in

bringing out the latent picture on an exposed plate.

It is less clear whether tissues react essentially as units, perhaps as a result of regional vascular or metabolic changes, or whether each cell responds separately as an individual. This problem has broad significance particularly in the case of the nervous system. Many behavior patterns, e.g., mating behavior, are dependent on sex hormones (see Beach, 1950) much in the same way as amphibian metamorphosis depends on thyroid. The fact that metamorphosis is accompanied by profound behavioral changes makes the parallel even closer. Yet the neurological basis of these hormonal effects on behavior is still quite obscure. It is reasonable to

TABLE 1

NUCLEAR SIZE OF MAUTHNER'S CELLS AFTER UNILATERAL TRANSECTION OF SPINAL CORD

CASE	AGE AT OPERATION	STAGE	DAYS POST-OPERATIVE	NUCLEAR DIAMETERS OF M-CELLS IN UNITS[a] EXPERIMENTAL	CONTROL
SA7[I]	33	V	5	9 × 7	9 × 6
SA7[II]	31	IV	5	10 × 7	11 × 6
SA8	31	IV	7	12 × 10	12 × 7
SA1	22	II	7	11 × 6	9 × 7
SA5	33	V	8	12 × 8	13 × 9
SA3[I]	43	VII	9	11 × 7	11 × 7
SA3[III]	43	VII	9	11 × 6	12 × 6
SA4	43	VII	9	12 × 7	10 × 7
SA6[III]	31	IV	11	11 × 6	10 × 9
SA5[I]	33	V	12	13 × 7	11 × 9
SA11[I]	33	V	28	11 × 8	15 × 8
SA15[II]	31	IV	28	14 × 7	12 × 7
SA12[II]	31	IV	29	10 × 6	10 × 8
			Av.	11.3 × 7.1	11.1 × 7.4
				80.2	81.4

[a] 1 unit equals 1.75 μ. The final averages are given as products of the average largest and smallest diameters.

assume that it consists of the selective and differential response to hormone of certain neuron systems, in contradistinction to others, based on innate differences among the respective neurons. The following account presents a crucial demonstration of such critical differences among neuron types in their response to the metamorphosing hormone.

The Test Object: Mauthner's Cell (M-Cell).—The hind brain of the tadpole contains, at the level of the entrance of the vestibular nerve, a single pair of giant cell bodies, so-called Mauthner's cells (hereafter referred to as "M-cells") with large descending axons generally considered to be concerned with swimming movements of tail and trunk. For further details

about these cells, we may refer to two recent reviews (Stefanelli 1950, 1951). During the larval period, the M-cells can easily be told from all other neurons of the surroundings by their extreme size. After metamorphosis, evidently correlated with the loss of undulatory swimming,

TABLE 2

MITOTIC ACTIVITY IN NORMAL HIND BRAIN

a	b	c	f	g	h
CASE	AGE AT FIXATION	STAGE	MITOTIC COUNT HIND BRAIN	MITOTIC COUNT SPINAL CORD	MITOTIC INDEX, HIND BRAIN
NP2[8]	33	V	18	20	11.3
NP2[8]	33	V	12	5	7.5
NP2[9]	43	VII	20	27	11.1
NP2[10]	43	VII	17	15	9.4
NP3	64	XIII	19	..	9.0
				Av.	9.7
NP4[III]	91	XXII	2	..	0.8
NP4[IV]	93	XXIII	0	..	0.0

they undergo regression,[1] losing size until they can no longer be distinguished. Our experiments were designed to analyze the causes of this regression.

According to recent studies (summarized in Young, 1950 and Weiss,

TABLE 3

MITOTIC COUNT IN HIND BRAINS WITH GRAFTS OF SUBMAXILLARY GLAND

a	b	c	d	e	f	h
CASE	AGE AT OPERATION	STAGE	STAGE AT FIXATION	DAYS POST-OPERATIVE	MITOTIC COUNT	MITOTIC INDEX HIND BRAIN
			FRESH SUBMAXILLARY			
FS1	36	V	V	3	27	16.4
FS2	36	V	V	3	10	5.9
FS3	39	VI	VI	3	10	5.8
FS4	39	VI	VI	3	10	5.8
FS5	39	VI	VI	3	14	8.2
FS6	39	VI	VI	3	12	7.0
					Av.	8.2
			BOILED SUBMAXILLARY			
BS1	34–36	IV–V	V	3	22	13.8
BS2	34–36	IV–V	IV–V	3	13	8.1
BS3	34–36	IV–V	IV–V	3	37	25.2
BS5	34–36	IV–V	IV–V	3	14	8.7
BS6	34–36	IV–V	IV–V	3	26	16.2
					Av.	14.8
					Grand Av.	11.2

1950a), neuronal size depends on trophic interactions with the peripheral tissues, perhaps through functional reinforcement (Hamberger and Hydén, 1945; Edds, 1950). These effects seem to operate, however, upon a background of intrinsically different growth potentials among different kinds of

neurons, emphasizing the multifactorial character of size determination. M-cells, specifically, have been shown to acquire their excessive growth capacity early in development (Rossetti, 1947; Stefanelli, 1951), though remaining subject to size variation in accordance with the extent of their dendritic fields. Nothing was known, however, about the factors causing their metamorphic reduction. Considering that neurons deprived of peripheral connections atrophy (Weiss, Edds and Cavanaugh, 1945; Sanders and Young, 1946; Cavanaugh, 1951), the M-cell regression could have been ascribed to the shrinkage of their peripheral field in the course of tail resorption. Failing this explanation, the next plausible assumption would be that the M-cell body itself reacts adversely to the metamorphosing

TABLE 4

MITOTIC COUNT IN HIND BRAIN AND CORD WITH THYROID GRAFTS

CASE	AGE AT OPERATION	STAGE	STAGE AT FIXATION	DAYS POST-OPERATIVE	MITOTIC COUNT: HIND BRAIN	MITOTIC COUNT: SPINAL CORD	MITOTIC INDEX, HIND BRAIN
T35[2]	34	IV	V	3	107	134	67.0
T34[2]	34	IV	V	3	78	35	49.0
T33[2]	34	IV	V	3	106	104	66.1
T32[2]	34	IV	V	3	74	149	46.1
T36[2]	35	IV	V	4	91	117	56.9
T18[I]	37	V	X	4	71	58	44.0
T39	41	V	VI	3	110	44	65.0
T18[III]	43	VI	XX	5	42	40	23.5
T27[I]	44	VI	XXII	6	45	44	25.0
T27[II]	44	VI	XXI	6	36	37	20.0
T27[III]	44	VI	XXI	6	55	...	30.5
T5[II]	45	VI	XXI	7	55	69	30.0
T5[III]	45	VI	XXI	7	52	50	29.0
T41[2]	46	VII	VIII	3	133	145	76.0
T26[I]	49	VII	XXI	6	17	14	9.0
T3[III]	58	VII	XXI	15	13	15	6.3
T28[II]	60	XI	XXI	5	42	20	19.9
T25[II]	62	XI	XX	7	25	8	11.8
						Av.	37.5

hormone. Experiments were therefore undertaken in which the effects on the M-cell of loss of tail innervation and of thyroid hormone could be tested separately, and both prior to, hence uncomplicated by, the normal metamorphosis of the test animal.

Materials and Methods.—Tadpoles of Rana pipiens, Xenopus laevis and Pseudacris versicolor were used. The former were staged according to Taylor and Kollros.

To test the loss of tail innervation, either tailless larvae were produced by removing the tail bud in the embryo, or the tail was later amputated and kept from regenerating by periodic reamputations, or, lastly, the spinal cord was hemisected in the second trunk segment, the anterior stump be-

ing turned upward to prevent reconnection of the longitudinal tracts containing the M-axon. The success of the operation was later checked histologically.

The effect of thyroid hormone on the nerve cells was tested by the technique developed in this laboratory by Kollros (1943), implanting a hormone source in the vicinity of the hind brain. We used either fragments of rat thyroid gland or flakes of agar ca. 0.5 mm. across, soaked for 20 minutes in a 1:1000 solution of thyroxin (synthetic "Roche Organon"). In control experiments, fragments of parotid gland of rats were used. The grafts were placed either into the fourth ventricle itself or on top of the choroid plexus.

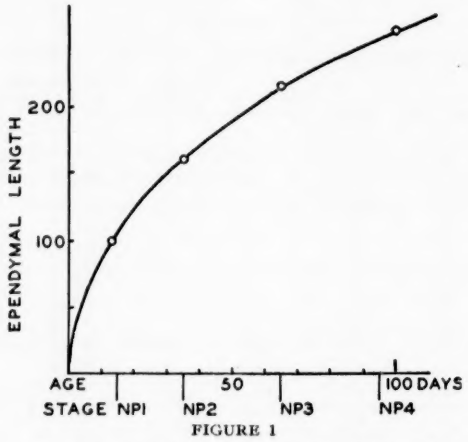

FIGURE 1

Growth curve of 4th ventricle at level of M-cells. Length of the ependymal lining in cross-sections of hind brain (in arbitrary units) plotted over larval age.

Most animals were sacrificed within the first week after the operation. They were fixed and stained either by Bodian's protargol technique or, for mitotic counts, by Heidenhain's iron hematoxylin. Brain cells were measured by projecting the slides on paper under high magnification, selecting in each preparation 100 cells at random from the vicinity of the M-cells and recording their nuclear diameters in equidistant size classes, as described previously for nerve fibers (Weiss, Edds and Cavanaugh, 1945; Cavanaugh, 1951). In M-cells, the longest and shortest nuclear diameters were measured, and in addition, the area of the largest cross-section of the whole M-cell body was determined by planimeter.

M-Cell Bodies after Amputation of M-Fibers.—Both tail amputation and high spinal cord severance deprived the M-fibers of a major portion of their

length and all the corresponding innervation area. Contrary to expectations, this trenchant reduction of peripheral load left the size of the cell bodies essentially unaffected.

Tail Amputation: Tails (15–25 mm. in length) were amputated in 6 tadpoles (28–36 mm. total length). The 12 M-cell nuclei of these animals (1, 3 and 6 days postoperative) measured 82.7 square units on an average, while 12 M-cells of normal control animals of comparable age and size (tail length 20–23 mm., total length 30–35 mm.) averaged 81.6 square units. Thyroid treatment, as will be shown later, produces its most marked effects on cell size during the first week. The fact that tail amputation had no effect whatsoever during the corresponding period is therefore significant.

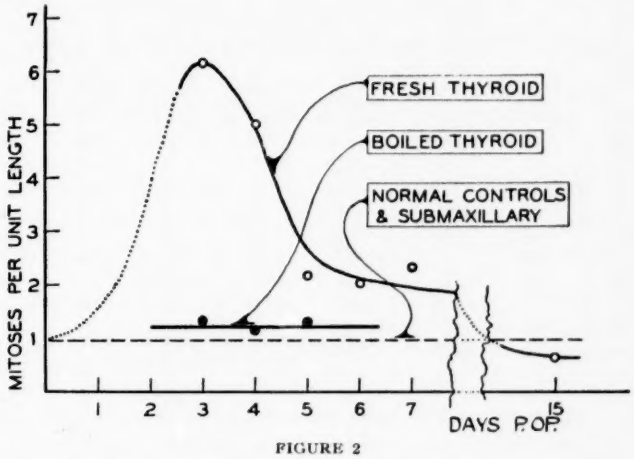

FIGURE 2

Mitotic index in hind brains of controls (broken line) and experimental cases (solid lines).

Spinal Cord Hemisection: Table 1 summarizes the measurements of M-cells in 13 animals preserved at various intervals after unilateral cord section. Of the two M-cells of each animal, one ("control") has an intact fiber with normal peripheral distribution, while the other ("experimental") has only a fiber stump. Yet both cells have remained about equal in size up to 29 days after the operation, averaging 81.4 and 80.2 square units, respectively, which is nearly the same value as in the preceding series.

In conclusion, amputation of a major portion of an M-fiber by either method has failed to cause regression of the cell body within the period of investigation. The Nissl pattern of the cell bodies was likewise found to

have remained unaltered. These facts seem to prove that, contrary to Bodian's (1947) assumption, the mere loss of axonal substance does not of itself cause neuronal atrophy. Such atrophy, where it occurs, is rather the result of the loss of functional connections (Weiss, 1950). In the present case this would lead one to suspect that the residual collateral connections of the M-fiber stump or newly formed connections at the level of transection may have been sufficient to preserve the integrity of the neuron.

At any rate, these results rule out the idea that M-cells regress after metamorphosis because the resorption of the tail has deprived them of innervation territory. We therefore turned to the second alternative, that of a direct response of the M-cell body to the metamorphosing hormone of the thyroid.

TABLE 5

MITOTIC COUNT IN HIND BRAIN WITH BOILED THYROID GRAFTS

a	b	c	d	e	f	g	h
					—MITOTIC COUNT—		
CASE	AGE AT OPERATION	STAGE	STAGE AT FIXATION	DAYS POST-OPERATIVE	HIND BRAIN	SPINAL CORD	MITOTIC INDEX, HIND BRAIN
TC42	52	IX	IX	4	30	20	19.0
TC43	55	X	XII	4	9	..	4.4
TC48	48	VIII	XI	3	36	22	18.0
TC46	48	VIII	VIII	3	7	..	3.5
TC49	58	XI	XIII	3	48	34	23.0
TC35	60	XI	XV	5	45	19	21.5
TC50	58	XI	XII	3	18	..	8.6
TC38	60	XI	XIII	5	27	32	12.8
TC32	60	XI	XV	5	15	12	7.2
TC40	60	XII	XIII	5	8	..	3.8
TC31	60	XI	XIII	5	34	10	16.2
TC30	60	XI	XIII	5	36	19	17.1
						Av.	13.0

Effect of Thyroid Grafts on Local Brain Growth.—A. Mitotic Effect: During metamorphosis, brain growth takes a marked spurt accompanied by intensified mitotic activity. Mitoses are confined to the ependymal layer lining the brain ventricles, and must therefore be referred to the number of nuclei in that layer rather than to the total number of brain cells. Being densely packed, the ependymal cells cannot be counted with the desired accuracy. But since the packing is uniform, the number of cells per unit length of ependymal lining is rather constant, hence mitotic rate can be expressed, for comparative purposes, as the number of mitoses per unit length of the ventricular contour. Thyroid grafts accelerate the local growth of the adjacent brain parts. In comparing their mitotic rates with those of normal tadpole brains, this expansion must be taken into account. For this purpose, the normal growth curve of the inner contour of the fourth ventricle in cross-section (at the M-cell level) was secured from a

series of measurements in normal samples stages (Fig. 1). From this curve the ependymal length, i.e., the denominator for the mitotic index, could then be read directly for any given age.

The mitotic data are summarized in tables 2–5 and figure 2. In view of the precipitate metamorphosis of their head region, animals with thyroid grafts were staged (column d) according to head criteria. In each case all mitoses in 10 alternate sections through the hind brain (column f) and 10 alternate sections through the spinal cord about 500 μ farther caudally (column g) were recorded. The total count amounts to 2335 mitoses.

Table 2 lists mitotic activity in normal controls for the age group used in the experiments. Omitting the last two stages (XXII and XXIII), during which mitotic activity has practically ceased as a result of maturation, an average mitotic index (stages V through XIII) of 9.7 is calculated. Table 3 gives the mitotic counts in animals of comparable ages which had received implants of fresh or boiled rat parotid glands in or near the fourth ventricle, 3 days after the operation, which is the time when the mitotic effect of thyroid grafts (see below) reaches its peak. The table shows that neither the operation as such nor the presence and discharges of a foreign gland of similar consistency as thryoid have any marked effect on mitotic activity, the average index (11.2) being not much above that of unoperated controls. This average of the pooled data has been used as control base in figure 2 (dotted line).

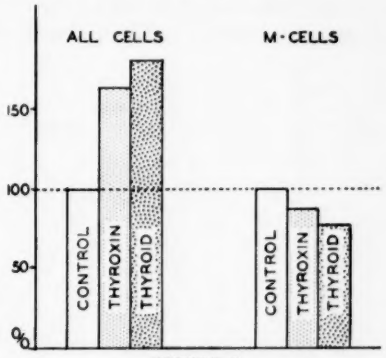

FIGURE 3

Relative volumes of non-M-cells (left) and M-cells (right), with controls set at 100%. Each non-M bar represents 1000 cells.

By contrast, table 4, summarizing the cases with thyroid grafts, shows a marked elevation of the mitotic index, particularly during the early days following the operation. On the third day postoperative, the mitotic index reaches an average (6 cases) of 61.5, which is about 600% of the normal and control indices. From this peak the index gradually declines (Fig. 2), until by day 15 it has returned to a normal level. Comparison of columns c and d shows that external features of the animals have advanced by several stages, indicating a regional acceleration of metamorphic events by the thyroid discharge from the grafts. Thus by the sixth day postoperative the various heads showed characters of regular stage XXI–XXII larvae. In view of the fact that the mitotic index at such late stages would normally

already be very low (less than 1; see table 2), the average of 22.2 for the seven animals counted on days 6 and 7 postoperative is still extremely high. It is difficult, therefore, to decide how much of the decline of the curve in figure 2 is due to the dissipation of the hormone source as a result of the disintegration and phagocytosis of the graft, and how much to the precipitate maturation, hence depletion of germinal cells, of the ependymal layer. But the fact that hormone delivery from the graft is of limited duration will be further supported below.

In the brains with thyroid grafts, an occasional extra-ependymal mitosis was observed, but these seem to have been true germinal cells which had simply been crowded out from their ventricular sites during division.

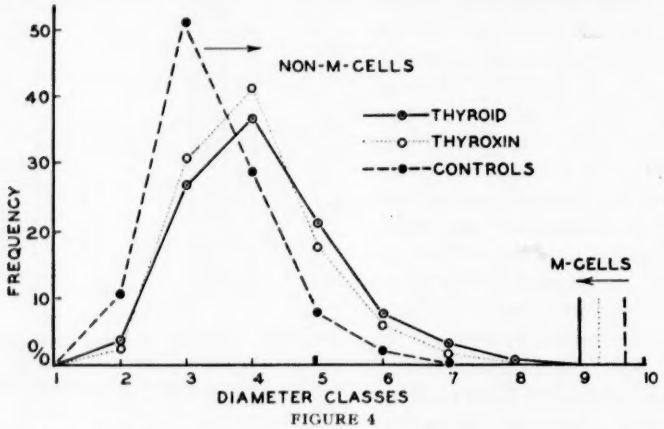

FIGURE 4

Histograms of nuclear diameters in 1000 non-M-cells each of control, thyroid and thyroxin cases. Mean diameters of M-cells for corresponding series are also indicated.

Table 5 gives the mitotic counts in the vicinity of grafts of boiled rat thyroid. The average index of 13.0 (Fig. 2) is only slightly higher than that of non-thyroid controls (tables 2 and 3). Even if the five ostensibly negative cases (TC32, 40, 43, 46 and 50) are omitted, the remaining average of 18.2 is still considerably lower than the average of 51.4 calculated from table 4 for cases with live thyroid grafts of comparable postoperative ages. Boiled thyroid thus seems to have had a perceptible, but weak, mitotic effect. Since it is generally recognized that thyroid hormone as such does not lose potency in boiling, the different effectiveness of fresh and boiled glands in our experiments must presumably be attributed to differences in the mode of release. Either the cells of the fresh gland continue to produce hormone even after grafting, or, more likely, the boiled gland has been so altered in

the process of boiling and coagulation that hormone will not be as readily released, especially since the boiled cells, in contrast to live ones, will not undergo autolysis.

The mitotic effect of thyroid grafts placed near the fourth ventricle spreads far down into the spinal cord. Table 4 reveals mitotic counts similar to those of the hind brain (column f) at a cord level 500 μ behind (column

TABLE 6

DISTRIBUTION OF NUCLEAR SIZES IN HIND BRAIN SAMPLES OF RANA PIPIENS

CASE	AGE OR STAGE (DAYS)	DAYS POST-OPERATIVE	NUCLEAR DIAMETERS IN UNIT CLASSES 1	2	3	4	5	6	7	8	MEAN NUCLEAR DIAMETER OF M-CELLS, $d_m = \sqrt{d_{max} \cdot d_{min}}$	
Controls												
NP3[II]	64		1	6	48	25	13	7	0	0	8.8	9.8
NP2[9]	43		0	11	41	39	6	2	1	0	..	..
NP2[10]	43		0	9	52	28	9	2	0	0	9.5	9.8
NP2[6]	33		0	1	45	38	12	3	1	0	9.9	9.4
NP2[II]	33		0	2	56	32	6	4	0	0	9.4	10.0
NP2[8]	33		1	8	54	27	9	0	1	0	7.9	8.8
NP2[III]	33		0	22	55	17	6	0	0	0	..	..
NP2[IV]	33		0	11	44	36	6	3	0	0	11.4	10.8
NP2[3]	31		1	22	53	19	5	0	0	0	10.0	..
NP2[1]	31		0	14	53	27	5	1	0	0	9.9	9.2
Av.	38		0.3	10.6	50.1	28.8	7.7	2.2	0.3	0	Av. = (see text)	9.7
Total relative volume 4822 cu. units.												
Thyroid Grafts												
T39	38	3	0	1	14	35	33	12	4	1	7.3	9.4
T41	43	3	0	1	17	43	22	8	9	0	9.8	9.2
T6[I]	38	13	0	6	31	44	10	8	1	0	9.5	9.2
T8[I]	33	8	0	6	34	42	13	5	0	0	8.9	8.8
T6[II]	38	13	1	8	55	25	7	4	0	0	8.5	9.9
T7[II]	33	10	0	4	26	31	21	14	4	0	8.8	12.2
T3[II]	43	9	0	2	24	42	24	5	3	0	8.1	8.4
T18[I]	33	4	0	2	17	35	26	11	5	4	8.8	8.8
T5[II]	38	7	0	3	26	32	28	6	4	1	9.4	8.5
T36	31	4	0	1	25	40	28	5	1	0	8.0	7.8
Av.	37		0.1	3.4	26.9	36.9	21.2	7.8	3.1	0.6	Av. =	9.0
Total relative nuclear volume 8821 cu. units.												
Thyroxin-Agar Grafts												
C5[II]	II–IV[a]	6	0	4	35	41	13	4	3	0	9.5	9.4
C5[I]	II–IV	6	0	1	22	42	24	10	1	0	8.8	9.4
C13[III]	VI–IX	7	0	4	32	35	16	9	4	0	8.1	11.4
C13[IV]	VI–IX	7	0	2	26	38	22	9	3	0	9.4	8.8
C1	V	4	0	2	24	53	18	3	0	0	8.5	7.9
C7	V	6	0	3	30	35	18	9	5	0	8.8	8.9
C12	VIII–IX	8	0	2	33	41	17	6	1	0	8.8	9.0
C3	V–VIII	6	0	3	33	42	14	6	1	1	10.5	10.5
C11[2]	V	4	0	3	36	43	16	2	0	0	10.2	7.9
C13	V–IX	4	0	3	38	40	15	4	0	0	9.9	8.9
Av.			0.0	2.7	30.9	41.0	17.3	6.2	1.8	0.1	Av. =	9.3
Total relative nuclear volume 7650 cu. units.												

[a] Indicates head stages at beginning and end of experiments.

g). To obtain a more complete picture of the spread, two normal larvae (33 days old; stage V) and two larvae with thyroid grafts (36 days, stage V, and 46 days, stage VII), the latter 3 days after implantation, were sectioned frontally, and all mitoses in alternate sections of the spinal cord were counted. The counts were, for the normal animals, 462 and 662 mitoses (average, 562), and for the ones with grafts, 1840 and 1180 (average, 1510)

mitoses, revealing a two- to threefold increase above normal. Since according to table 4, a higher increase was found at a far anterior cord level, it is evident that the effect grades off anteroposteriorly within the cord. Presumably it spreads by diffusion from the ventricle through the central canal.

B. Effect on Cell Size: While the ventricular cells near thyroid grafts grew and divided, the cells of the mantle, which had lost their mitotic capacity, just grew in size. Table 6 lists 10 cases with thyroid grafts, 10 cases with thyroxin-agar implants and 10 normal controls of comparable average ages; in each case, a random sample of 100 nuclei from the vicinity of the M-cells was taken and their distribution according to diameter classes recorded (the nuclei being nearly spherical). From these figures, the total relative volume of the 100 nuclei was calculated according to the formula:

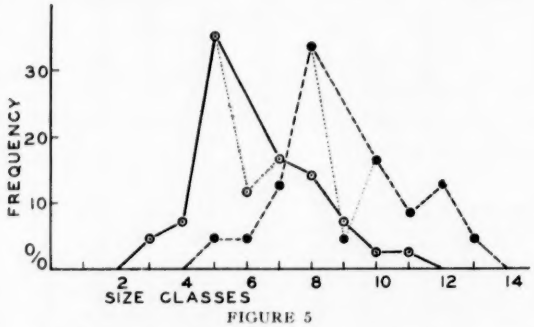

FIGURE 5

Histograms of M-cells (largest cross-sectional areas) in controls (- - ● - -) and thyroid- or thyroxin-grafted cases (—⊙—) of Rana pipiens.

$V = M_1 + 2^3M_2 + 3^3M_3 + 4^3M_4 + \ldots + n^3M_n$; where M_1, M_2, M_3, etc., represent the numbers of nuclei recorded in size classes 1, 2, 3, etc. The mean diameter of each ellipsoid nucleus of the M-cells was determined as the square root of the product of the longest times the shortest diameters. The relative volumes of the M-nuclei are given as the cubes of the mean diameters. The resultant changes in nuclear volumes are summarized in the graph, figure 3, as percentages of the normal control values. The nuclei of non-M-cells have gained 59% with thyroxin-agar grafts, and 83% with thyroid grafts, while the nuclei of the M-cells of the same animals have lost 12% and 19%, respectively. It is uncertain whether the lower effectiveness of the synthetic, as compared to the natural, product is a matter of potency or merely of dosage. But it is evident that thyroid hormone in either form affects the sizes of M-cells and of non-M-cells in opposite directions.

This antagonistic response of the two neuron types suggests some fundamental difference in their constitution. On the other hand, it is equally conceivable that the growth stimulation by thyroid is merely an inverse function of cell size, reversing its sign at a critical size level above that of the largest non-M-cells, but below that of the M-cells. This possibility, however, is ruled out by the fact that all size classes of non-M-cells are about equally enhanced. Figure 4 shows the histogram of nuclear diameters for the non-M-cells in the three groups of animals listed in table 6. It can be seen that the thyroid treatment has moved the whole nuclear population up by approximately one size class; the larger size classes are, if anything, favored. The graph also gives for comparison the mean diameter values of the M-cell nuclei, showing the displacement in the reverse direction from that of the histograms of non-M-nuclei.

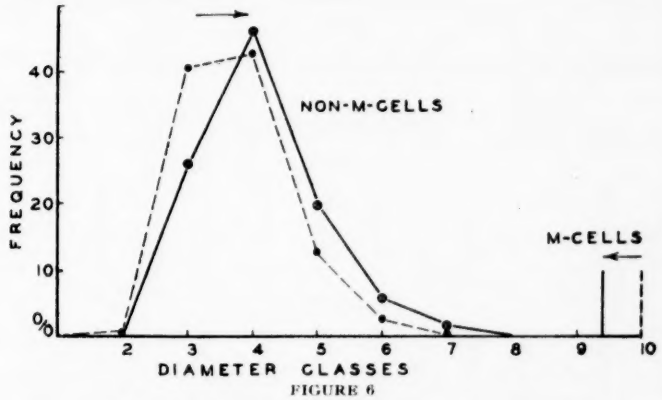

FIGURE 6

Histograms of non-M-nuclei in normal (- - ● - -) and thyroid-grafted cases (—⊙—) and record of the mean diameters of the M-nuclei (Xenopus laevis).

Histograms for the M-cells were made from planimetric measurements of the largest cross-sections of the cell bodies rather than from nuclear measurements; the greater accuracy of this method seemed indicated in view of the fact that the size of the sample of M-cells was only one-fiftieth of that of non-M-cells. Values for thyroid- and thyroxin-cases have been pooled. The resulting histograms for 24 normal (- - ● - -) and 43 experimental (— ⊙ —) cells are reproduced in figure 5; the size classes are on a different scale than those in figure 4. It is immediately evident from the graphs that thyroid treatment has affected all M-cells about equally, regardless of size, causing a shift of the whole population downward by about 3 size classes. These data prove clearly, particularly when taken in conjunction with those of figure 4, that the populations of M-cells and of non-M-cells

react as wholes in qualitatively different fashions, and that cell size as such is not a decisive factor.

The average volumes of M-cell bodies, computed from the planimetric data, were in the thyroid series approximately 60% of those of the control series. Comparing this value with the corresponding reduction of nuclear volumes to ca. 80% (Fig. 3), it appears that Bok's formula (1934), according to which the volumes of cell bodies of neurons vary as the squares of their nuclear volumes, holds roughly in the present case, too. It has likewise been found to apply to the atrophy of ganglion cells following peripheral disconnection (Cavanaugh, 1951).

All experiments described in the foregoing were done in Rana pipiens. An additional series with grafts of rat thyroid was carried out in the faster growing South African clawed toad, Xenopus laevis. The results, compiled

TABLE 7

DISTRIBUTION OF NUCLEAR SIZES IN HIND BRAIN SAMPLES OF XENOPUS LAEVIS

CASE	AGE AT FIXATION	DAYS POST-OPERATIVE	NUCLEAR DIAMETERS IN UNIT CLASSES 1	2	3	4	5	6	7	8	MEAN NUCLEAR DIAMETER OF M-CELLS, $d_m = \sqrt{d_{max.} \cdot d_{min.}}$	
Controls												
X1C	27		0	1	49	35	12	2	1	0	10.5	9.6
X4C	27		0	1	42	46	7	3	1	0	9.8	9.9
X1C1	27		0	1	43	42	11	3	0	0	10.5	10.5
X4C1	27		0	0	40	46	12	2	0	0	9.4	9.0
CX5	31		0	1	31	48	19	1	0	0	10.5	10.4
CX5-6	31		0	0	37	41	15	5	2	0	10.0	10.2
Av.			0	0.66	40.3	43.0	12.6	2.6	6.6	0	Av. = 10.0	
Thyroid Grafts												
XII2	20	5	0	0	37	47	12	3	1	0	8.8	9.9
X4[3]	22	5	0	0	38	42	12	7	1	0	8.8	9.2
X5-7	24	8	0	0	29	41	20	7	3	0	9.4	9.9
X5-6	24	8	0	0	26	41	19	12	2	0	8.9	9.4
X1[1]	17	10	0	0	15	55	25	4	1	0	8.9	
X4[3]	22	5	0	0	21	43	28	7	1	0	9.9	10.4
X1[1]	17	10	0	0	22	54	21	1	2	0	8.8	9.2
Av.			0	0	26.9	46.1	19.6	5.9	1.6	0	Av. = 9.4	

in table 7 and figure 6, are quite similar to the previous ones. The thyroid hormone produced an increase in size of the non-M-cells, but reduction in the size of the M-cells (loss of M-nuclear volume: 17%). The histograms of the M-cells bodies based on the largest cross-sectional areas are given in figure 7, showing the shift of the thyroid-treated M-cells toward the smaller size classes.

Residual Effects.—The observed acceleration of proliferation and cell growth naturally is reflected in dimensional changes of the whole cross-section of the hind brain. While the processes thus far discussed augment the gray matter, concomitant outgrowth and presumably caliber increase of nerve fibers from the enlarged brain parts add to the thickness of the white matter. The resulting over-all changes are summarized in the graphs,

figures 8–10. Figure 8 represents the normal "growth curve" of the cross-section of the hind brain during the larval period, based on planimetric measurements of the total cross-section, as well as of the gray matter only, in 23 normal controls. Figure 9 shows the increase in cross-sectional area in the medullae oblongatae containing thyroid grafts. As one can readily see, growth is initially accelerated, falling off by the 6th day, in general agreement with the detailed cell events discussed above. In figure 10, finally, the growth curves of the thyroid cases and of normal controls of comparable ages (taken from Fig. 8) are combined. These curves show quite conclusively that the thyroid-induced growth acceleration has been only temporary. After the initial rise, the curves for the experimental animals run strictly parallel to those for the controls. This means that the former retain their erstwhile advance, but otherwise continue to grow at a normal rate once the thyroid source has been exhausted. The action thus

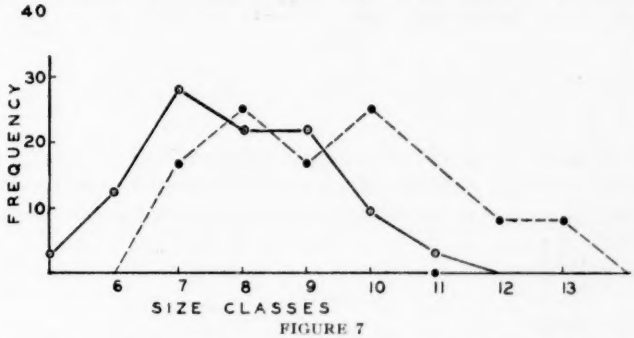

FIGURE 7

Histograms of the M-cell bodies in control (- - ● - -) and thyroid graft cases (Xenopus). Note opposite shift from that in figure 6.

is comparable to that of a single solid dose of hormone administered over a limited period, and the course of events clearly proved that the observed growth phenomena were direct, rather than secondary, consequences of the local hormone action.

Discussion.—The local hormone application in these experiments has but precipitated events which eventually would have taken place in the course of normal metamorphosis. In this regard, the results merely amplify earlier observations on circumscribed metamorphic changes in the vicinity of thyroid grafts (e.g., Hartwig, 1940; Kollros, 1943). In fact, Kollros, *et al.*, in a recent preliminary note (1950), report size increase in midbrain cells in response to thyroxin pellets implanted in to the cranial cavity, quite similar to the response of the non-M-cells of the hind brain in our own experiments.

The added significance of our results lies in the demonstration of the fact

that different neurons of the same locality, the M-cells and the non-M-cells, respond in wholly different ways, resulting in changes of opposite sign. Again, regression of M-cells amidst growing non-M-cells would have been

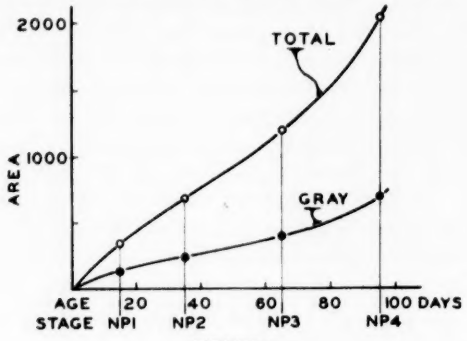

FIGURE 8

Cross-sectional increase (area in arbitrary units) of hind brain and its gray matter in normal larvae up to 100 days.

a normal feature of these brains during metamorphosis anyhow; but we now have proof that while the change as such is brought on by the thyroid hormone, the pattern of the change is predetermined in the responding cells,

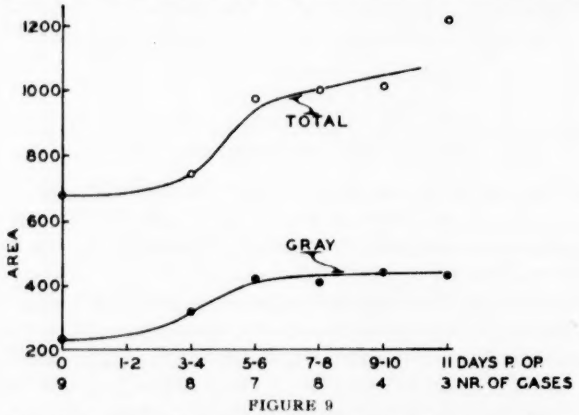

FIGURE 9

Growth of cross-section of hind brain following thyroid implantation at approximately stage NP2 of figure 8.

which evidently constitute a mosaic of quite diverse cell species. Thus, both the property of M-cells to respond to thyroid by involution, and the property of non-M-cells to respond to the same agent by growth, are but at-

tributes of the more distinctive constitutional differentials acquired by these various cell types previously in the course of embryonic differentiation. Having been dealt with comprehensively in some recent publications (Weiss, 1949, 1950), these problems need not be labored here further.

Two points, however, should be reemphasized. First, since the differently reacting cell types lie intimately intermingled, their differential response cannot be attributed to differential exposure to, and accessibility by, the hormonal pool. Second, since the non-M-cells of all sizes have been equally enhanced in their growth (figs. 4, 6), the degrowth of the M-cells

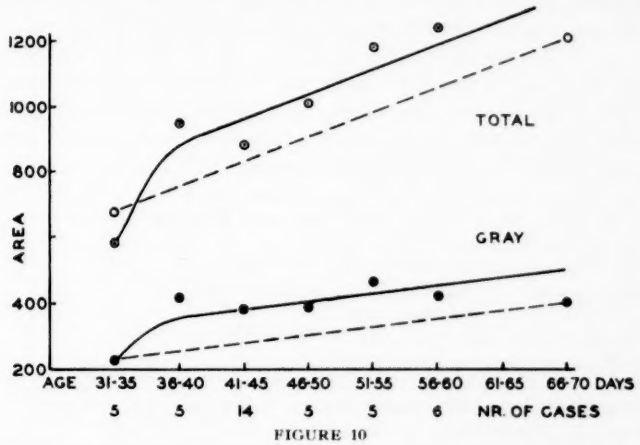

FIGURE 10

Growth curves of cross-section of hind brain in normal (broken lines) and thyroid graft cases (solid lines).

cannot be explained simply by their large size, that is, by mere quantitative extrapolation of the positive growth effect beyond a critical upper size level. The difference between the two cell types thus is not merely one of degree, but one of kind, and the large size differential between M-cells and non-M-cells is but one of the superficial signs of much more profound constitutional, i.e., physicochemical and metabolic distinctions. It is reasonable to assume that similar distinctions exist among the non-M-cells, except that either they do not become reflected in cell size or our techniques of measurement and group assay are too crude to detect them. Indirect evidence for the qualitative differentiation ("speciation") among neurons is accumulating rapidly; numerous examples have been cited in the recent symposium on Genetic Neurology (Weiss, 1950b). The evidence pertains both to the fact that specific qualitative differences among neurons exist and to the relevance of these differences to the establishment and maintenance of functional order in the nervous system. Their relevance to the insuring of de-

velopmental order, in counterplay with the humoral milieu, is demonstrated clearly by the results of this paper. Whether this also brings us any nearer to an understanding of the relation between hormone action and patterns of behavior remains to be ascertained.

Summary.—Grafts of rat thyroid or thyroxin-agar implants near the hind brain of tadpoles produce within a few days a burst of mitotic activity in the neural epithelium and marked growth of nerve cells with the exception of Mauthner's neurons, which atrophy. Evidence is presented to show that this antagonistic response, which occurs similarly in normal metamorphosis in response to the metamorphosing hormone of the thyroid, is based upon qualitative differences among the neuron types involved. The data confirm the view that hormones do not create differentials among equipotential tissue units, but merely help to realize preexisting latent differentials. The results stress anew how relevant qualitative diversity among neurons is to the development of neural order, and perhaps suggest a clue to the mechanism of hormonal "control" of behavior.

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[1] A brief note that has come to our attention after this manuscript had gone to press (G. M. Baffoni and G. Catte, *Atti della Accad. Naz. dei Lincei*, 8th ser., vol. **9**, fasc. 5, pp. 282–287 (1950)) repor's precipitate involution of Mauthner's cells in tadpoles kept in water with dilute thyroid extract.

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